RESEARCH GROUP: Northern Ireland Centre for Stratified Medicine

Project Title:
Molecular genetics of B cell chronic lymphoproliferative disorders.

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Level: PhD

Background to the project:
Multiple myeloma (MM), is an incurable haematological malignancy of end stage B lineage cells, or plasma cells (1). It has an incidence of approximately 65 new cases per million of the population per annum, with a male:female ratio of 1.3:1. Differences in incidence are seen in different ethnic groups. Overall survivals have improved recently from a median of 3–4 years to 5–7 years, although upwards of 25% of patients survive for less than 24 months. Major obstacles to improved outcomes are the disease's heterogeneity, drug resistance and the immunosuppressive nature of the tumour in its bone marrow microenvironment. Monoclonal gammopathy of undetermined significance (MGUS), from which most, if not all, cases of myeloma are thought to evolve, has an incidence of 3-4% in the over 50 age group. Approximately 1% of cases of MGUS progress to PCM per annum. Smouldering myeloma (SMM) constitutes approximately 14% of all cases of myeloma and carries a 10% risk per annum of progression to MM(2). Factors contributing to progression of these conditions to MM are unknown and there is an urgent need to improve understanding of their biology and identify biomarkers to enable prediction of disease progression, and responsiveness to specific treatments (1,3).

Objectives of the research project:
This is a longitudinal study, with serial sampling of patients at different time points. Named collaborators include NHS clinicians caring for the patients. Significant preliminary findings include identification of tumour plasma cells in peripheral blood (PB) in approximately half the patients tested to date, including some with MGUS and some MM patients considered to be in complete remission (CR) using conventional criteria. Using multicolour flow cytometry, these cells are sorted and stored for future molecular analyses. Cellular and serum markers will be analysed on all patients, at each sampling time-point, to improve understanding of the clinical immunosupression that occurs. These include lymphocyte subsets in PB and BM, pro- and anti-inflammatory cytokines, HevyLite chain analyses and scrutiny of the uninvolved serum Ig levels.

Most previous studies have used cell lines, or single patient samples only allowing a snapshot at one time-point. Serial sampling will facilitate analyses of tumour cells from presentation/diagnosis through to the almost inevitable relapse and development of drug resistance in terminal stages. This will contribute to improved understanding of proliferation, survival and drug resistance factors involved in disease progression in individual patients, the pathways involved, and may identify novel therapeutic targets for future drug development.

Specific molecular analyses planned include: SNP analyses of MDR genes using genomic DNA, IGHV mutational status and gene usage, next generation sequencing (NGS), gene expression profiling (GEP), iFISH (all using tumour derived nucleic acids) and microRNA (miRNA) analyses using PB. These molecular investigations have been designed:

1. To verify our earlier findings that MDR SNPs contribute to multidrug resistance and poorer outcomes using a larger cohort of patients.
2. To test the hypothesis that somatic hypermutation of IGHV genes has taken place in the tumour cells following antigen presentation and class switching (demonstrated by <98% homology with germline).
3. To determine if commonality of IGHV gene usage is seen in MM, as we and others have reported in chronic lymphocytic leukaemia, a B cell malignancy occurring at an earlier stage of B cell ontogony.
4. To perform NGS, GEP and iFISH on serial samples, to identify and monitor changes in genetic aberrations present at diagnosis and occurring during disease progression. Such analyses should allow identification of key pathways involved in proliferation, survival and drug resistance.
To perform miRNA analyses in PB, using a targeted approach. This is particularly pertinent as miRNAs are likely to have a significant role in novel treatments within the next few years.

**Methods to be used**:
Centre for Stratified Medicine will be used to identify and sort tumour plasma cells. A minimum of eight fluorochrome labelled monoclonal antibodies will be used to differentiate between normal and tumour plasma cells. The sorted cells will be stored at -80 degrees Celsius for subsequent nucleic acid extraction to be used for the molecular analyses. Flow cytometry will also be used for lymphocyte subset analyses.

**Cytokine analyses**:

**Protein studies**: Serum protein electrophoresis, kappa and lambda free light chain quantification and ratios, and HevyLite analyses, using kits supplied by The Binding Site (Birmingham, England), will be performed in the Altnagelvin Hospital Clinical Biochemistry Laboratory in liaison with the Biomedical Scientist responsible for these assays.

**iFISH**: this will be performed under the direction of Dr Sheila O’Connor, HMDS, Leeds.

**NGS & GEP**: these will be performed either in-house, or outsourced, depending on anticipated purchase of equipment, or otherwise.

**miRNA analysis**: this will be performed using serum samples and utilising a targeted approach.

**Miscellaneous laboratory methods**: these include laboratory procedures well established in the Centre, including density gradient cell separation, AutoMacs cell separation using monoclonal antibody labelled microbeads, and nucleic acid separations.

**Skills required of applicant**:
It is not expected that applicants will have all the laboratory skills required. Training will be given. More important are the following:

- Good academic ability, as evidenced by a 2.1 or first class honours degree in biomedical or associated science,
- Enthusiasm, flexibility and willingness to work outside conventional ‘office’ hours, as may be necessary to process patient samples,
- Good computing skills and knowledge of relevant software packages including Word, Excel, SPSS, PowerPoint, or their equivalents,
- Good interpersonal and communication skills, including the ability to give oral and poster presentations at scientific and medical meetings,
- A proven ability to tackle, and complete, challenging experimental tasks, and oral and written communications,
- An interest in cellular and molecular investigations in oncology, in particular haematological oncology,
- Knowledge of the innate and adaptive immune systems, and the components thereof.

**Scripting/programming in Linux/Shell, Python and R.**

Good statistical knowledge.

**References**:

