MYC translocation in aggressive B-cell lymphomas: why partner matters?

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Principal supervisor's CRUK CC theme: Haematological Malignancies Programme

Department for student registration: Pathology
Department or institute where research will take place: Division of Molecular Histopathology, Department of Pathology, Box 231 Level 3 Lab Block, Addenbrooke's Hospital, Hills Road

Postgraduate scheme:

- **MRes + PhD (1 + 3-year non-clinical applicants)**
- **Part-time MRes + PhD (2 + 3-year clinical applicants)**

MRes project outline:

MYC translocation is seen in ~10% of diffuse large B-cell lymphoma (DLBCL), a common and aggressive B-cell malignancy. Among MYC translocation positive DLBCL, IG::MYC accounts for ~55% of cases, while the remaining cases are non-IG::MYC but poorly defined for translocation partners. MYC translocation may occur together with BCL2 and/or BCL6 translocation. The clinical outcome of patients with DLBCL carrying MYC and BCL2 dual translocations, known as double-hits, varies considerably, and the cases with IG::MYC show significantly inferior survival than those with non-IG::MYC [1-4]. Intriguingly, only ~45% of DLBCL with MYC translocation show a high (>70%) MYC protein expression [5,6]. It is unclear why the prognostic effect of MYC translocation is confounded by its translocation partner and why MYC protein is not highly expressed in each case with MYC translocation? We investigated these important questions and our preliminary data show:

1) A significant proportion of MYC translocations in DLBCL involves BCL6 as its translocation partner;
2) Non-IG::MYC translocations including those with BCL6 as translocation partner have variable genomic configurations that may or may not transactivate MYC expression.

These findings suggest that not every non-IG::MYC translocation is a “driver” event, causing high MYC expression and consequently impacting on clinical outcome. We wish to expand these findings by study of additional DLBCL with non-IG::MYC translocation at both genomic and transcript levels.

MRes experimental plan:

1) Delineation of the genomic configuration of non-IG::MYC translocations by next generation sequencing (NGS). Selected cases of DLBCL with non-IG::MYC translocations will be investigated by NGS to delineate their genomic configuration and their potential to transactivate MYC expression. The translocation breakpoints will be mapped, partner genes identified, and their genomic configuration will be scrutinised for evidence (promoter substitution and/or presence of super enhancers) of potential transcriptional activation.

2) Correlation of non-IG::MYC translocation with MYC expression. In parallel, the above cases will be investigated for MYC expression by reverse transcription qPCR, RNAscope in situ hybridisation and immunohistochemistry. MYC expression level will be correlated with the potential of MYC transactivation by non-IG::MYC. Cases with IG::MYC as well as those without MYC translocation will be used as a reference.

PhD project outline:

MYC translocation is seen in ~10% of diffuse large B-cell lymphoma (DLBCL), a common and aggressive B-cell malignancy. Among MYC translocation positive DLBCL, IG::MYC accounts for ~55% of cases, while the remaining cases are non-IG::MYC but poorly defined for translocation partners. MYC translocation may occur together with BCL2 and/or BCL6 translocation. The clinical outcome of patients with DLBCL carrying MYC and BCL2 dual translocations, known as double-hits, varies considerably, and the cases with IG::MYC show significantly inferior survival than those with non-IG::MYC [1-4]. Intriguingly, only ~45% of DLBCL with MYC translocation show a high (>70%) MYC protein expression [5,6]. It is unclear why the prognostic effect of MYC translocation is confounded by its translocation partner and why MYC protein is not highly expressed in each case with MYC translocation? We investigated these important questions and our preliminary data show:

1) A significant proportion of MYC translocations in DLBCL involves BCL6 as its translocation partner;
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These findings suggest that not every non-IG::MYC translocation is a “driver” event, causing high MYC expression and consequently impacting on clinical outcome.

We wish to expand these findings by study of additional DLBCL with non-IG::MYC translocation at both genomic and transcript levels, and test the hypothesis whether a combined analysis of MYC translocation and MYC expression can be reliably used for identification of “driver” MYC translocation. Eventually, we will establish an evidence-based pathway for application of MYC translocation as a prognostic marker in routine clinical service.

PhD experimental plan:

The primary research question is whether a combined analysis of MYC translocation and MYC expression can be used for reliable classification of non-IG::MYC translocation into activation (“driver”) and non-activation (“passenger”) event, and whether such classification can transcribe into clinically distinct prognostic groups, i.e. DLBCL with non-IG::MYC(driver) are similar to those with IG::MYC, while cases with non-IG::MYC(passenger) resemble those without MYC translocation. This will be achieved through the planned investigations as outlined below.

1) Delineation of the genomic configuration of non-IG::MYC translocation in DLBCL and investigate its transactivation potential (promoter substitution and/or presence of super enhancers) by NGS; We aim to investigate ~100 cases of DLBCL with non-IG::MYC translocation.

2) Correlation of the transactivation potential of non-IG::MYC translocation with MYC mRNA/protein and MYC target genes expression; MYC expression will be investigated by reverse transcription PCR, RNAscope in situ hybridisation and immunohistochemistry, while

MYC target gene expression will be assessed by revisiting our archival data or RNAseq if not available.

3) Classification of non-IG::MYC translocations into “driver” or “passenger” event according to the level of MYC and MYC target gene expression using cases with IG::MYC translocation as a benchmark;

4) Validation of the above classification in both clinical trial and population based cohorts: The molecular (GCB, MHG, ABC), genetic (EZB, MCD, etc) subtypes and clinical outcome will be compared among IG::MYC, non-IG::MYC(driver) and non-IG::MYC (passenger) groups in both clinical trial (REMoDL-B, MaPLe) and population-based cohorts (HMRN, HODS-Cambridge);

5) Establishment of an optimal and integrated pathway for application of MYC translocation and MYC protein expression as a prognostic marker in routine clinical service.

Main techniques:

Next generation sequencing to investigate chromosome translocation and mutation profile, and related data and bioinformatic analysis;

Interphase in situ hybridisation to investigate chromosome translocation /structural alterations;

Reverse transcriptional quantitative PCR, RNAscope in situ hybridisation and immunohistochemistry to investigate MYC expression;

Various statistical analyses of clinical and laboratory data.

Key references:

1. Copie-Bergman C, Cuillière-Dartigues P, Baia M, Briere J, Delarue R, Canioni D et al. MYC-IG rearrangements are negative predictors of survival in DLBCL patients treated with immunochemotherapy: a GELA/LYSA study. Blood. 2015;126:2466-74.

2. Rosenwald A, Bens S, Advani R, Barrans S, Copie-Bergman C, Elsensohn MH et al. Prognostic Significance of MYC Rearrangement and Translocation Partner in Diffuse Large B-Cell Lymphoma: A Study by the Lunenburg Lymphoma Biomarker Consortium. J Clin Oncol. 2019;37:3359-68.

3. Sha C, Barrans S, Cucco F, Bentley MA, Care MA, Cummin T et al. Molecular High-Grade B-Cell Lymphoma: Defining a Poor-Risk Group That Requires Different Approaches to Therapy. J Clin Oncol. 2019;37:202-12.

4. Cucco F, Barrans S, Sha C, Clipson A, Crouch S, Dobson R et al. Distinct genetic changes reveal evolutionary history and heterogeneous molecular grade of DLBCL with MYC/BCL2 double-hit. Leukemia. 2020;34:1329-41.

5. Ziepert M, Lazzi S, Santi R, Vergoni F, Granai M, Mancini V et al. A 70% cut-off for MYC protein expression in diffuse large B cell lymphoma identifies a high-risk group of patients. Haematologica. 2020;105:2667-70.

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