Long-read RNA sequencing enables integrated genomic risk stratification and reveals isoform-level drivers of high-risk multiple myeloma in the UK MRA RADAR study

Anandagopal Srinivasan[1], Bethan Hudson-Lund[2], Eleanor Calcutt[1], Emma Lyon[2], Jessica Kimber[2], Alice Lefever[2], Annabel Laidler[2], Elise Rees[2], Amatta Mirandari[2], Maximilian Goodman[3], Megan Hawksley[3], Mohammad Kazeroun[1], Naser Ansari-Pour[3], Matthew Jenner[4], Laura Chiecchio[5], Martin Kaiser[6], Michael Chapman[7], Angela Hamblin[1], Kara-Louise Royle[8], Karthik Ramasamy[9,10], Kwee Yong[2,11], Eileen M Boyle[2], Sarah Gooding[1,12], Adam Cribbs[1]

1. *University of Oxford, Oxford Translational Myeloma Centre, NDORMS Botnar Research Centre, Oxford, United Kingdom,*
2. *University College London, UCL Cancer Institute, London, United Kingdom,*
3. *Oxford University Hospitals NHS Foundation Trust, Regional Genetics Laboratory, Oxford, United Kingdom,*
4. *University Hospital Southampton, Department of Haematology, Southampton, United Kingdom, University Hospital Southampton,*
5. *Wessex Genomics Laboratory Service (Salisbury), Southampton, United Kingdom,*
6. *The Institute of Cancer Research, London, United Kingdom,*
7. *University of Cambridge, Department of Haematology, Cambridge, United Kingdom,*
8. *University of Leeds, Leeds Cancer Research UK CTU, Leeds, United Kingdom,*
9. *University of Oxford, Oxford Translational Myeloma Centre, NDORMS Botnar Research Centre, London, United Kingdom,*
10. *Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom,*
11. *University College Hospital, London, United Kingdom,*
12. *University of Oxford, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom*

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Background: Current IMWG risk stratification in multiple myeloma (MM) relies on genetic events detected by FISH and/or DNA sequencing but fails to identify all poor risk cases. Gene expression profiling (GEP) improves prognostication yet requires additional assays and does not resolve isoform diversity. Long-read RNA sequencing (LR-RNAseq) offers simultaneous detection of structural variants, gene expression, and alternative splicing. We evaluated whether LR-RNAseq could provide integrated genomic and transcriptomic risk assessment.

Methods: Newly diagnosed MM patients from the UKMRA RADAR trial and Oxford HaemBiobank (n=103) underwent Nanopore-based LR-RNAseq with UMI-based quantification, DNA profiling (Myeloma Genome Panel) and FISH defined IMWG-2025 risk classification. RNA-based detection of copy number alterations (CNA) and translocations was benchmarked against DNA, focusing on risk-defining structural variants. Published, validated GEP risk signatures were extracted from RNASeq as previously described. Isoform discovery and differential splicing analyses were performed.

Results: LR-RNAseq accurately detected key CNAs (sensitivity 0.67, PPV 0.93) and translocations (sensitivity 0.89, PPV 1.0). Overall agreement with DNA-based IMWG-2025 risk classification was 83%. Discordant cases were largely due to *TP53* mutations and deletions detectable only by DNA-based assays due to low *TP53* gene expression. In 61 patients with day 100 post-autograft MRD assessment, LR-RNAseq-derived IMWG risk profiling demonstrated comparable predictive performance to FISH/MGP, with similar sensitivity (0.29 vs 0.32) and modestly higher PPV (0.50 vs 0.42). The 2 MRD+ve patients classed high-risk by DNA but not RNA both had *TP53* mutations. Notably, a combined LR-RNAseq-based model integrating IMWG and established GEP-based risk assignment improved prediction of MRD positivity (sensitivity 0.77, PPV 0.57), indicating complementary prognostic information captured by transcriptomic and genetic risk features. LR-RNAseq uniquely identified novel splice isoforms in key myeloma drivers, including *TP53* and *KRAS*, which were significantly enriched in high-risk disease across independent classification systems (e.g., *TP53*: OR 5.2 in ISS III; *KRAS*: OR 4.49 in GEP-HR). Differential isoform usage was observed in biologically important genes including *BCL2*, a key mediator of venetoclax sensitivity in MM.

Conclusion: LR-RNAseq captures established genomic risk features while enabling GEP application and revealing potentially relevant isoform-level biology. In our cohort, integrated RNA-based risk models outperformed established DNA- and RNA-based approaches in predicting residual disease. This unified, cost-effective, single-assay approach has potential to streamline current molecular diagnostics while uncovering novel drivers of high-risk MM. Ongoing follow-up and cohort enlargement will determine the prognostic and therapeutic relevance of isoform-based stratification.