A review of IGHV gene analysis in Waldenström's Macroglobulinaemia and the potential survival advantage of cases with IGHV3-23 gene rearrangements

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Background

Molecular characterisation of Waldenström's Macroglobulinaemia (WM) has advanced in recent years. However, where IGHV mutational status has been explored extensively in other lymphoproliferative diseases, such as Chronic Lymphocytic Leukaemia (CLL), there have been relatively few cohorts investigated in WM, with the largest published dataset (excluding MGUS) being only 58 cases.

Aims & Methods

- To determine IGHV gene use and MYD88 L265P mutation status in 43 WM cases from the Royal Bournemouth Hospital (RBH) diagnosed between 1993 and 2016. RBH cohort characteristics are summarised in table 1.
- To combine and analyse RBH IGHV data with the 5 largest previously published datasets giving a total cohort size of 226 cases (Figure 1).
- To investigate time to first treatment (TTFT) in the RBH cohort based on utilisation of IGHV genes, using IBM SPSS Statistics 22 software to generate Kaplan and Meier survival curves and median TTFT .

Table 1: Patient Summary	RBH (n=43)
Male/Female	29/14
Median age at diagnosis years (range)	74 (48-89)
Median follow-up months (range)	61.6 (1-209)
No. of treated cases	27/35 (7 Unknown)
IgM paraprotein g/L (range)	29 (2.6-80) (14 unknown)
No. of cases with IGHV gene sequence analysis (aligned using IMGT database)	43
No. of cases with MYD88 L265P MLPA analysis (MRC Holland PO38)	41

Figure 1. Geographical locations of the largest published datasets

[1]Rollett, RA. *et al*. Clin Lymphoma Myeloma. 2006 Jul;7(1):70-2

[2]Walsh, SH. *et al*. Leuk Res. 2005 Jul;29(7):729-34

[3]Petrikkos, L. *et al*. Biomed Res Int. 2014;2014:809103

[4]Varettoni, M. *et al*. Leuk Lymphoma. 2013 Nov;54(11):2485-9

[5]Martín-Jiménez, P. *et al*. Haematologica. 2007 May;92(5):635-42

Results

In the largest analysed cohort to date (n=226; Table 2) we confirm the significant over-representation of IGHV3 family genes and in particular IGHV3-23 compared to both normal PB (CD5-/CD5+) and CLL IGHV rearrangements (IGHV3; p=0.0076 and p=0.0003 (Figure 2a) and IGHV3-23; p=0.0279 and p=0.0042 respectively (Figure 2b)).

Table 2: RBH data & published datasets	UK (n=43)	Greece (n=35)	Sweden (n=15)	Spain (n=58)	Leeds, UK (n=20)	Italy (n=55)
	RBCH	**Petrikkos *et al***	**Walsh *et al***	**Martin-Jiminez *et al***	**Rollett *et al***	**Varettoni *et al***
% IGHV3 of total (no)	69.77 (30)	74.29 (26)	53.33 (8)	75.86 (44)	65 (13)	87.27 (48)
% IGHV3-23 of total (no)	25.58 (11)	25.71 (9)	13.33 (2)	29.31 (17)	20 (4)	25.45 (14)

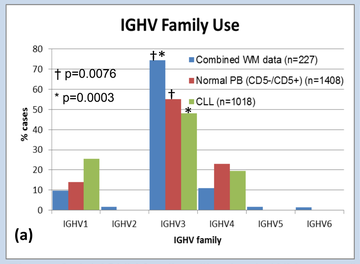

Figure 2. (a) IGHV family utilisation in WM cases (blue) compared with most frequently utilised families in normal PB (CD5-/CD5+) (red) (Stamatopoulos *et al*, 2007) and CLL cases (Davis *et al*, 2016) (green); (b) Significant difference in IGHV3-23 utilisation in WM (blue) compared with normal PB (red) and CLL cases (green)

RBH IGHV3-23 sequences were further analysed in detail;

- median % identity for IGHV3-23 cases - 91.16% (range 86.18%-93.06%), all cases 91.36% (range 86.18%-99.31%)
- no evidence of stereotypy
- median CDR3 length for IGHV3-23 cases -15 amino acids (AAs) (range 8-19), all cases -14 (range 10-23)
- mutations were clustered between AAs 55 and 63 and within mutation hotspots (figure 3)
- AA 55 was replaced in all cases; changing from a hydrophobic AA to a neutral AA (10/11) or a hydrophilic AA (1/11)
- 13 superantigen recognition sites were retained in 8/11 IGHV3-23 cases

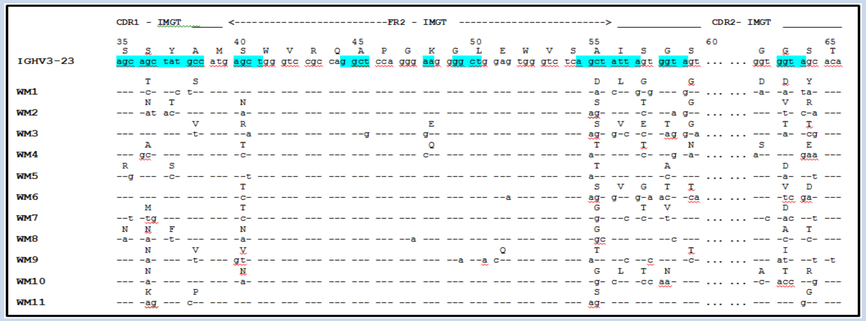

Figure 3. Highlighted in blue are IGHV3-23 mutation hotspot motifs wa - (a/t)a, tw - t(a/t), rgyw - (a/g)g(c/t)(a/t), wrcy - (a/t)(a/g)c(c/t).

Outcome data was then assessed for RBH cases; median follow-up for all RBH cases was 61.6 months (range 1- 209 months) and available median TTFT was 10.1 months (95% CI; 6.9-13.2 months). When TTFT was assessed based on IGHV gene use there was a trend for an increased TTFT for IGHV3-23 cases compared to all other IGHV rearrangements (61.6 months vs. 9.6 months; p=0.058) (Figure 4a) and when compared to other IGHV3 only family genes this reached significance (61.6 vs.2.9 months; p=0.018) (Figure 4b).

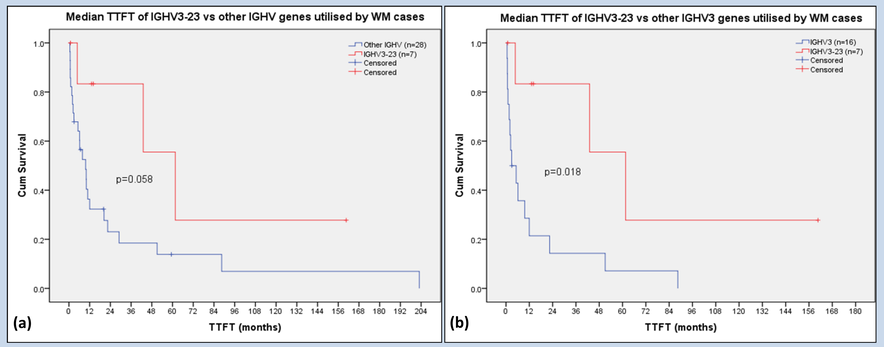

Figure 4. (a) TTFT for RBH IGHV3-23 cases compared with all other IGHV genes utilised; (b) TTFT for RBH IGHV3-23 cases compared with only IGHV3 genes utilised.

In addition to IGHV analysis, MYD88 L265P mutation detection was performed on 41/43 RBH cases and detected in 36/41 (87.8%) patients, this is comparable to 14 previously published cohorts (range 65% and 100% MYD88 positive). Of potential note, where corresponding MYD88 mutation and IGHV data was available (n=70), all IGHV cases with ≥98% identity (n=5) were MYD88 negative.

Conclusions

In the largest number of cases analysed as a single cohort to date (n=226) we have:

- confirmed the significant overrepresentation of IGHV3 gene usage and specifically IGHV3-23 in WM patients
- we extend the potential association between cases with IGHV gene identity ≥98% and negative MYD88 L265P

Within the RBH cohort (n=43) we:

- identified possible recurrent/shared mutations in IGHV3-23 cases
- noted a potential treatment free survival advantage for IGHV3-23 cases when compared to other IGHV3 family and all other IGHV genes.

Larger collaborative studies with outcome data are necessary to confirm these findings.

Acknowledgements

There are no relevant conflicts of interest to disclose.