

## [ABSTRACT WM1.2]

### TRACKING THE MALIGNANT CELL OF ORIGIN IN WALDENSTROM'S MACROGLOBULINEMIA

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*Background.* Waldenstrom's macroglobulinemia (WM) is characterized by an IgM-expressing lymphoplasmacytoid lymphoma that infiltrates the bone marrow (BM). Typically, WM displays a morphological spectrum, from small B-lymphocytes to maturing cells with CD138 expression. Disease outcome in WM varies, with survival spanning 5-10 years. Factors determining outcome are as yet not known, but characterizing tumor biology in WM will be of value in understanding these. A central question in WM biology has been aimed at defining the cell of origin giving rise to disease and its clonal history. Most studies here have relied on the analysis of immunoglobulin (Ig) variable (V) genes in tumor cells, providing important insights. This arises from the role that V gene encoded determinants play in normal B-cell survival, where they allow antigen recognition via the B-cell surface receptor (BCR). Cognate antigen can trigger somatic mutation (SM) in the germinal center (GC) in secondary lymphoid organs, during normal B-cell development, for which the enzyme activation induced cytidine deaminase (AID) is a critical requirement.<sup>1</sup> A further modification of the BCR can also occur in the GC, when effector function is altered by isotype class switch recombination (CSR), again dependent on AID activity.<sup>1</sup> This involves double strand DNA breaks and recombination of isotype specific genes in the IgH locus on chromosome 14q32. Normal B-cells that exit the GC with mutated V genes circulate as memory B-cells, and generally express CD27.2 sIgM+ve memory B-cells can also home to the BM.

#### **Mutational status V genes**

In WM, tumour-derived V genes revealed SM in early small cohort studies, with no intraclonal variation in sequences between tumour clones, consistent with neoplastic origins from a post-follicular B-cell.<sup>3,4,5</sup> Furthermore, analysis of isotype switch variants appeared to indicate that arrest occurs prior to switch events,<sup>3</sup> apparently substantiated by findings that switch events in WM are impaired *in-vivo*.<sup>5</sup> However, as the numbers of cases examined has increased, some WM tumours have emerged which display unmutated (UM) VH genes,<sup>4</sup> indicating origins from a naïve, pre-GC cell of origin. Although the extent of UM WM is as yet not known, it clearly indicates that disease origins in WM are heterogeneous. To probe disease presentation further in WM, we evaluated VH gene features by contrasting cohorts where cDNA had been extensively amplified (ampcDNA) to increase sensitivity with cases where cDNA was prepared conventionally. <sup>6</sup> Overall, 16/16 cases revealed mutated (MUT) VH genes, suggesting that the UM subset may be a minor component in WM. Mutational status in WM, however, could potentially be an important predictor of outcome, as defined in IgM-expressing chronic lymphocytic leukemia (CLL), where the UM subset has a profoundly poorer outcome.<sup>7</sup>

## CSR

Surprisingly, we observed tumour-derived isotype switch variant transcripts in WM using amp-cDNA. Tumour V(D)J-C $\gamma$  co-existed with C $\alpha$  (6/7 cases), and with C $\gamma$  (3/7 cases) transcripts, with assays indicating a low frequency.<sup>6</sup> Sterile germline transcripts and switch circle transcripts, generated from excised switch circle DNA, and the hallmark of deletional CSR, could also be identified. *AID* transcripts were also present, indicating a low level of CSR events *in vivo* in WM. These observations were substantiated in WM cases in which cDNA had not been amplified (3/9 cases), with data also pointing to limitations of small WM cohort studies that can clearly overlook tumour-associated events occurring at subclonal levels.<sup>3,4,5</sup> Some WM cells however, have also been shown to undergo isotype switch *in vitro* following appropriate stimuli.<sup>5</sup> Deletional CSR with *AID* expressed poses a potential risk to the genome, and may underlie recurrent abnormal chromosomal translocations in the IgH switch region. In multiple myeloma (MM), IgH switch region (SH) translocations are frequent clonal events, revealing isotype switch as an important stage in disease origins.<sup>8</sup> In contrast, this does not appear to be the case in MUT WM. Although CSR events occur in some WM cells, they are subclonal and appear not to trigger abnormal 14q32 genomic events, as these are not found in the vast majority of cases analyzed.<sup>9</sup> In this regard, parallels exist with CLL,<sup>7</sup> where although UM cases express both switch transcripts and AID, they display little evidence for any switched Ig expression or SH chromosome 14q32 abnormalities, negating their role in pathogenesis, as in WM.

## CD27 expression

Another feature that impacts on deciphering origins of WM is the expression of the post-GC marker, CD27,<sup>2</sup> also found on normal IgM-expressing memory B-cells. Variable CD27 expression in MUT WM has been proposed as indicative of origins of disease from an unusual memory B-cell that bypasses the GC.<sup>5</sup> To assess this, WM tumour cells were analyzed at a sub-population level.<sup>6</sup> In 2/2 cases, mutated tumour cells were identifiable in both CD27+ive and CD27-ve fractions, confirming heterogeneous CD27 expression within an otherwise monoclonal tumour. The question arises whether this is due to origins from a cell lacking CD27 and undergoing ectopic SM, with tumour cells then acquiring CD27, or whether MUT WM derives from a CD27+ post-GC memory B-cell which progresses to loss of CD27 expression. On-going, possibly ectopic, mutations with *AID* expressed can certainly occur in CD27-ve tumour cells, as we have shown in hairy cell leukemia,<sup>10</sup> whereas intratumoural loss of CD27 in MM associates with advancing disease, possibly related to escape from CD27-CD70 apoptotic signals.<sup>11</sup> Furthermore, the existence of CD27-ve memory B-cells remains controversial, and data from our current work to address this in relation to WM will be presented at the IVth IWWM meeting, 2007.

## Intratumoral diversification in WM

Isotype switched tumour cells could be tracked further in some WM single cells from both CD27+ve and CD27-ve fractions, at a low frequency (3/45 cells).<sup>6</sup> Switch activity therefore remain a feature of the evolving clone as it losses (or gains) CD27 expression. In single WM cells, none were found to co-express V(D)J-C $\beta$  and V(D)J-C $\alpha/\gamma$ ,

confirming deletional CSR events. The expression of *AID* in some pre- and post-switch cells also raises the possibility that it could catalyze abnormal gene modifications. Excised DNA during CSR, as can occur in WM, has a potential for destabilizing gene expression via re-insertion as transposons. Interestingly, when the pattern of SM was compared in single WM cells, there was unexpected evidence for on-going somatic mutation in V(D)J-C $\lambda$  sequences, in both CD27+ve and CD27-ve fractions.<sup>6</sup> AID was also identifiable in CD27 $\pm$ ve cells, suggesting on-going ectopic mutational events, occurring at a low frequency. Initiation of SM in normal B-cells requires antigen signaling via sIg, and although most WM tumour cells will express sIgM, it is at present not known whether this plays a role in mediating signals to initiate SM, or indeed what the nature of the antigen might be. It is also as yet not defined whether on-going mutations in WM are associated with a blast phase, as is seen in normal GC B-cells, and what role this may have in feeding the tumour population. It does however indicate that the BM is conducive to mutational activity. A striking feature of lymphoma cells that undergo continual SM, and remain in the GC site, is their ability to generate novel N-glycosylation motifs via mutated nucleotides in V genes. These are functional and in follicular lymphoma appear mandatory, suggesting a role in tumor-stroma interactions.

<sup>12</sup> Given that localised mutations can be identified in MUT WM, we examined a series of 14 cases for such sites, using paired VH and VL analysis. In these, and in a further 7 WM VH genes, the incidence of glycosylation sites was at a low, background level. SM in these WM therefore appears not to lead to acquisition of glycosylation sites. Such modifications are also absent in MUT CLL. These findings indicate no relationship between WM and GC lymphoma tumors. Instead, location in BM and heterogeneity in SM activity further point to a closer similarity to CLL of tumour behaviour at this site. Of note, proliferating centres in CLL are localised to the BM.<sup>7</sup> It is most likely that a low level of switching in some WM cells occurs as post-transformation events, since a cell of origin that is continuously doing so would invariably generate a marked IgG/A component in this disease on a frequent basis, and this is uncommon. As these and mutational events are on-going in some WM cells, they do raise the possibility that a degree of clonal expansion could occur on a few occasions, but that in general either disease associated or disease-stroma associated factors appear to advantage the IgM-expressing clone. However, this could be compromised on rare occasions, and may in fact underlie recent observations in a case study of WM, where although the patient presented with a typical IgM only spike, switched tumour-derived progeny comprising 41% of tumour cells together with 57% of the persisting IgM clone could be detected 4 years later.<sup>13</sup> Interestingly, in that study rituximab therapy ablated the IgM+ clone, but the IgG+ subclone persisted, raising the scenario that CSR events in this case may also have generated further genetic hits to potentiate survival.*Concluding comments.* In summary, these observations are consistent with the concept of neoplastic arrest in most WM as occurring in a mutated IgM-expressing memory B-cell, which retains the capacity to undergo a low level of SM and isotype switching in some cells following transformation. *This work was supported by the Leukaemia Research Fund (UK), and in-part by the MMRF (USA)*

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