Preclinical characterisation and development of a novel myelodysplastic syndrome-derived cell line

Myelodysplastic syndrome (MDS) represents a group of heterogeneous haematopoietic disorders characterised by diverse clinical symptoms varying from mild anaemia to multilineage cytopenia.[1,2] Importantly, one-third of MDS patients will exhibit a transformation towards acute myeloid leukaemia,[3,4] increasing the complexity of the disease aetiology. Over the last decade, our understanding of the molecular pathogenesis of MDS was greatly increased by the development of several genetically and epigenetically modified mouse models.[5,6] However, a specific clinically relevant humanised MDS mouse model to evaluate the efficacy of new therapies is lacking.[7–9] Efforts to develop xenograft strategies have also been frustrated by misassignment of MDS cell lines (i.e. ofthe 31 MDS cell lines published to date, only one has been determined to truly represent MDS).,[10] The MDS92 cell line, described by Tohyama *et al.*,[11] has been confirmed to have an MDS phenotype[10] and originated from a bone marrow isolate of a 52-year-old man with refractory anaemia (RA) exhibiting ring sideroblasts. Subsequent investigations confirmed the presence of typical MDS aberrations such as 5q and 17p deletion, monosomy 7, a complex karyotype,[10,12] and clonal heterogeneity evidenced by MDS92's ability to generate several subblast derivatives, including MDS-L.[12] Following its initial reporting,[13] the MDS-L cell line has become a standard preclinical tool for MDS therapeutic development,[7–9] culminating in the establishment of a reproducible and widely used preclinical MDS model.[8] However, a study by Kida *et al.*[12] recently demonstrated that MDS-L exhibits two major clones, termed MDS-L-2007 and MDS-LGF, where the MDS-LGF cells can proliferate with low addition of IL-3 (1 ng/ml), and MDS-L-2007 requires higher supplementation of IL-3 (100 ng/ml). Given the significant use of the MDS-L cells in MDS preclinical research,[7,14,15] it is critical to delineate and characterise whether both clones, independently or cumulatively, in the xenograft setting contribute to MDS towards development of appropriate preclinical models of MDS.

To evaluate the engraftment potential of the two cell lines, we performed extensive preclinical experiments in the NSG and NSGS mouse strains. Both cell lines were transduced with a dual reporter gene [luciferase and green fluorescent protein (GFP)] permitting longitudinal bioluminescence visualisation of *in vivo* cell engraftment. Thus, 5×10^6 cells were injected intravenously into NSGS ($n = 5$) mice (Fig 1A). Clear evidence of engraftment was determined for the proposed MDS-L-2007 clone in all NSGS mice as early as week 4 with a progressive increase in bioluminescence (Fig 1A, B) in haematopoietic organs (Supplementary Figure S1A, B) leading to fatal haematologic disease, with an average latency of 55 ± 3 days (Fig 1C). MDS-L-2007 cells have also engrafted in NSG mice albeit with a longer disease latency (112 ± 6 days, data not shown). In contrast, MDS-LGF cells did not engraft either NSG or NSGS mice (in total $n = 89$ mice) by any route or precondition regimen tested (Supplementary Table SI). These results suggest that previously published xenografts of MDS-L might represent a specific subclonal engraftment of the MDS-L-2007 clone.[8] To further characterise the differences between MDS-LGF and MDS-L-2007 clones, we performed immunophenotyping of the two cell lines by mass cytometry (Supplementary Table SII). As shown in Fig 1D, we found major differences in CD7 and HLA-DR expression, confirming Kida *et al.*'s previous observations. However, our extended panel additionally identified aberrant expression of CD33 (highly expressed on the cell surface of MDS-LGF cells) and CD38 (highly expressed on the cell surface of MDS-L-2007 cells; Fig 1D). Interestingly, low CD38 expression is a characteristic found in most MDS patient $CD34^+$ cells.[16] Conversely, CD33 is frequently expressed on MDS cells[17] but its expression has not been found to correlate with clinical cytogenetics, therapy response or survival in MDS. However, as the biological function of CD33 has not been fully elucidated, its potential involvement in disease development cannot be excluded. Subsequently, fluorescence *in situ* hybridisation (FISH) analysis of chromosome 5q was performed on both cell lines. As shown in Fig 1E, F, the 5q deletion (5q31, EGR1) was observed in 82% of MDS-L-2007 cells with a log2 ratio of −0·321 by using a copy number variation (CNV) array (Supplementary Table SIII), although the log2 ratio in MDS-LGF was −0·187 and, surprisingly, below the probe cut-off (<10%). The presence of this aberration in MDS-L-2007 cells corresponds to results obtained in a previously established animal model using the MDS92 mother cell line.[8,10–12,18] Subsequently, we performed copy number arrays, G-banding and sequencing of 54 genes found to be frequently mutated in myeloid leukaemia. The results (Supplementary Table SIII) indicated that both cell lines present with complex karyotypes and mutations in several genes involved in the development of MDS (*NRAS, CEBPA* and *TP53*). The presence of these three mutations corroborates the previous observation by Kida *et al.*[12] In addition, the