Prognostic implications of MUC1 and XBP1 concordant expression in multiple myeloma: A retrospective study

Sheerien Rajput
Centre for Regenerative Medicine and Stem Cell Research, The Aga Khan University.
Khurram Minhas
Department of Pathology and Laboratory Medicine, The Aga Khan University.
Iqbal Azam
Department of Community Health Sciences, The Aga Khan University.
Usman Shaikh
Department of Pathology and Laboratory Medicine, The Aga Khan University.
El-Nasir Lalani (elnasir.lalani@aku.edu)
Centre for Regenerative Medicine and Stem Cell Research, The Aga Khan University.

Research Article

Keywords: MUC11, XBP12, myeloma3, prognosis4, cancer stem cells5.

Posted Date: April 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1557743/v1

Abstract

Background:

Multiple myeloma (MM) is a disease of malignant plasma cells (PC) with poor survival. Disease progression and treatment relapse are attributed to MM cancer stem cells (CSCs) and signaling molecules such as MUC1 and XBP1. The prognostic value of the expression of CSC associated biomarkers, MUC1 and XBP1 in MM, has not been explored previously

Method:

In this study, we determined the immunohistochemical expression of CSC markers (ALDH1, CD117 and CD34), MUC1 and XBP1in 128 MM formalin-fixed paraffin-embedded bone marrow archival blocks. The expression of biomarkers was assessed for association with clinicopathological variables and the overall survival of patients. Descriptive & initial univariate survival analyses were performed using Kaplan Meier curves. Univariate and multivariable analyses were performed using simple and multiple Cox regression models. The results are reported as crude & adjusted hazard ratios with 95% confidence intervals.

Results :

Expression of ALDH1 and CD117 was found in 51% and 48% of the tumors, respectively. ALDH1 expression was associated with 1.83 years of reduced survival for patients with CD56 negative tumors. MUC1 expression was observed in 62%, whereas XBP1 was expressed in 48% tumors. Combinatorial group analysis of XBP1 and MUC1 stratified patients into two prognostic groups. Cases with tumors negative for expression of MUC1 and XBP1 (XBP1- / MUC1-) were categorized as a ***good prognostic group*** with increased survival of 3.42 years compared to cases with tumors expressing both (***Worst prognosis***, XBP1+/MUC1+)**.** The adjusted hazard ratio showed a four-fold increased risk of mortality associated with the concordant expression of MUC1 and XBP1 in patients who were >65 years of age

Conclusion:

Concordant expression of MUC1 and XBP1 in MM defines a subset of patients with adverse outcomes. Of these, patients diagnosed at >65 years of age have a significantly higher risk of death.

1 Introduction

Multiple myeloma is an incurable disease of neoplastic plasma cells that inhabit the bone marrow (BM) for growth and survival. Genetic and epigenetic factors contribute to molecular and phenotypic variations, leading to inter and intra-tumoral heterogeneity. Prognostication of MM patients is challenging as patients present with varied clinical symptoms, stages of disease and responses to treatment [1, 2].

Over the last two decades, novel therapies have improved patients' overall survival from a few months to over a decade in some cases [3]. However, despite this progress, MM remains an incurable chronic

malignancy, and < 10% of the patients experience long-term survival [4, 5].

John Dick and Dominique Bonnet (1997) introduced the classical Stem cell model in the hematopoietic system. The model postulates that the whole mass of the cancerous cell population is dependent upon an undifferentiated, quiescent, and slow proliferating group of cells known as "Cancer Stem Cells" (CSCs) [6]. CSCs have the potential to recapitulate the original tumor along with multi-lineage differentiation potential, resistance to toxins, and unlimited proliferative capacity [7, 8]. Identifying, isolating, and understanding this biologically distinct subpopulation of cells is essential for developing targeted and effective therapies. The first evidence for a quiescent and drug-resistant population in MM came from Drewinko and colleagues [9]. Although the genetically distinct type of MM CSCs markers' expression and clinical relevance in patients with MM have not reached a consensus yet, independent or combinatorial expression of CD117, ALDH1, and CD34 are considered markers associated with the presence of MM-CSC-like cells.

MUC1 expression has been identified in 2–10% of normal human BM mononuclear cells, CD34 + cells, resting T cells, B cells, and plasma cells [10]. Aberrant expression of MUC1 has been demonstrated to play a seminal role in cancers, including MM [11–13]. MUC1 mediated nuclear translocation of β-catenin and NF-kB complex modulates tumor cell survival, anchorage-independent growth, and drug resistance in MM cells *in vitro* [14–16]. Bar-Natan and colleagues (2017) recently demonstrated that MUC1 expression was significantly up-regulated when MUC1 expressing MM cells were co-cultured with the HS5 stroma cell line. MM cells exhibited increased drug resistance and a high proliferative index in co-culture experiments. [17]. However, the prognostic value of MUC1 expression in predicting the outcome of patients with MM remains unclear [18, 19].

X Box Binding Protein 1 (XBP1) is a basic-region leucine zipper (bZIP) protein belonging to the CREB/ATF (cAMP response element-binding protein/activating transcription factor) family of transcription factors (TF). XBP1 expression is indispensable for plasma cell differentiation and immunoglobulin synthesis. There is significant evidence demonstrating the pathogenic role of XBP1 in cancers and its utility as a therapeutic target. Elevated levels of XBP1 have been shown to exhibit inverse prognostic significance in MM patients. Furthermore, high expression of XBP1 has shown a correlation with advanced disease and poor prognosis in Thalidomide-treated MM patients [20, 21]. The pathogenic role of XBP1 in MM was best described by Carrasco et al. (2007) when they induced XBP1 expression in transgenic mice, and all the mice developed MGUS with a resemblance to human disease. Also, within two years, 26% of the mice developed MM with increased secretion of monoclonal immunoglobulins [22]. Furthermore, XBP1 induced secretion of IL-6 supports the growth and survival of myeloma cells [23]. The clinical significance of XBP1 regarding treatment resistance and prognosis has not, to our knowledge, been reported to date.

To date, there have been a handful of studies from Pakistan on MM and none examining the expression of CSC markers (CD34, CD117, and ALDH1), MUC1, and XBP1 and their potential association with prognosis and survival. This study was undertaken to evaluate the expression of selected markers in a cohort of MM patients and determine their prognostic relevance in terms of the patient's overall survival.

2 Materials And Methods

Study Design & Sample Collection:

This retrospective cohort study was designed in compliance with the REMARK guidelines and "Declaration of Helsinki" [24–26]. The study was undertaken at the Aga Khan University Hospital (AKUH), Pakistan. AKUH is a not-for-profit university teaching hospital receiving referrals from across Pakistan. The study protocol was approved by the Ethical Review Committee of the AKUH (2741-Pat-ERC-13) and included 128 treatment-naive patients diagnosed with symptomatic MM.

Cases were identified from the MM registry maintained at AKUH and included diagnostic trephine biopsies of treatment naïve patients (see Supplementary Fig. 1**)**. A total of 307 MM patients were diagnosed and registered during the study period (from 2007 through 2015). Of these, 128 cases were selected based on the following criteria; a) availability of formalin-fixed paraffin-embedded (FFPE) tissue blocks, b) representative tumor tissue on hematoxylin eosin-stained sections. Study participants provided written informed consent to use the tumor tissues and their data for research.

Archival Formalin-fixed paraffin-embedded (FFPE) trephine tissue blocks were retrieved from the Department of Pathology and Laboratory Medicine, AKUH, and medical records were reviewed. Clinicopathological data such as gender, age at diagnosis, treatment, ISS stage, and follow-up details were recorded.

Immunohistochemical Expression:

Five-micron thick serial sections of FFPE trephine biopsies were cut and fixed onto poly-L-lysine coated slides to undertake IHC expression of ALDH1 (Fig. 1A), CD34 (Fig. 1B), CD117 (Fig. 1C), CD20 (Fig. 1D), CD45 (Fig. 1E), CD56 (Fig. 1F), CD138 (Fig. 1G), MUC1 (Fig. 1H) and XBP1 (Fig. 1I). Details of antibodies used are described in Supplementary Table 1.

Antibodies were diluted in Envision™ FLEX, Antibody Diluent (DM830). EnVision™ FLEX, Mouse, High pH (pH 9) kit by Dako (K8002) was used for tissue pretreatment and staining. Sections were dewaxed and processed for IHC as published previously [27]. Every fifth section was stained with hematoxylin and eosin to ensure the presence of representative tumor tissue (Fig. 1J).

Scoring for Immunohistochemical Expression:

Slides were reviewed by consultant hematopathologists who were blinded to the patient's clinical history. We utilized CD138 expression to identify plasma cells (PC). IHC expression was evaluated and scored for the proportion of cells expressing the respective biomarker. For ALDH1, CD117, and CD34 expression, the absence of expression (score = 0) was considered negative, and expression with scores > 0 was recorded as positive. A 10% cut-off was set for the rest of the markers, and expression in ≥ 10% of tumor cells was considered positive [27].

Statistical Analysis:

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics were computed as frequencies with percentages for the expression of immunophenotype markers and other variables. Mean and range are reported for quantitative variables such as age. Associations between the expression of markers and clinicopathological parameters were evaluated through the Chi^2 test. The number of patients for each variable varies since we did not have data for all factors for all study participants.

Date of event; in case of death, and date of last follow-up was taken as an endpoint for patient survival analysis. The prognostic value of biomarkers was assessed for association with overall survival (OS) using the Log-Rank test, and Kaplan-Meir curves were plotted. Simple and multiple Cox regression for univariate and multivariable analyses was used to identify the risk of mortality associated with the expression of markers and the resulting crude hazard ratios (HR) were reported with 95% confidence interval. All the variables with a *p*-value of < 0.2 were considered eligible for multivariable analysis. Multivariable analysis using multiple Cox proportional hazard regression were calculated for individual markers after adjustment of other markers. The results were reported as adjusted hazard ratio with a 95% confidence interval, and a p-value of < 0.05 was considered significant unless mentioned otherwise. Multicollinearity among eligible variables was also assessed.

3 Results

Patient Characteristics

Clinicopathological features of the patients are summarized in Table 1. Our study included 128 patients; of them, 88 were males. The mean age at diagnosis was 57 years (range 30–89 years). Based on the ISS staging system, 44% (n = 44/100) of the patients presented with stage III disease. IgG myeloma was the commonest immunoglobulin type. Almost 70% (n = 78/113) of the patients presented with boney lesions, while 51% of the patients (n = 52/101) had elevated levels of blood urea nitrogen (BUN) at the time of diagnosis. Impaired serum Beta-2 microglobulin was detected in 77.2% (n = 68/88) of the patients.

Table 1
Demographic Characteristics of Patients

Variable	Frequency	%	Median (Range)
Gender (n = 128)			
Female	**40**	**31.3**	
Male	**88**	**68.8**	
Age at Diagnosis (years) (n = 128)			
31–40	**9**	**7**	**57 (30–89)**
41–50	**24**	**18.8**	
51–60	**52**	**40.6**	
61–70	**25**	**19.5**	
71–80	**17**	**13.3**	
81–90	**1**	**0.8**	
Serum Immunoglobulins (Heavy Chain) (n = 118)			
IgG	**63**	**53.3**	
IgA	**19**	**16.1**	
Light Chain Only	**21**	**17.7**	
Non-Secretory	**15**	**12.7**	
Bone Lesions (n = 113)			
Absent	**35**	**31**	
Present	**78**	**69**	
BUN (n = 101)			
Normal (6–24 mg/dL)	**49**	**48.5**	**33 (7-118)**
High (MALE > 24, FEMALE > 21)	**52**	**51.4**	
Calcium Level (n = 113)			
Normal (8.6–10.2 mg/L)	**79**	**69.9**	
Low (< 8 mg/L)	**23**	**20.3**	**9 (5.3–15)**
High (> 11 mg/L)	**11**	**9.7**	
Serum Creatinine (n = 117)			
Normal (0.8–1.3 mg/L)	**51**	**43.5**	**1.3 (0.30–9.6)**

Variable	Frequency	%	Median (Range)
Low (< 0.8 mg/L)	12	10.2	
High (> 1.3 mg/L)	54	46.1	
Serum Beta-2 Microglobulin (n = 88)			
Normal (1–3 mg/L)	20	22.7	5.5 (0.33-47)
High (> 3 mg/L)	68	77.2	
Serum Albumin (n = 95)			
Normal (3.5–5.2 mg/L)	35	36.8	3.1 (1-5.1)
Low (< 3 mg/L)	60	63.1	
ISS Stage (n = 100)			
Stage 1	35	35	
Stage 2	21	21	
Stage 3	44	44	
Abbreviations: BUN (Blood Urea Nitrogen), Ig (Immunoglobulin), ISS (International Staging System)			

Only 17 cases of the study population were tested for chromosomal aberrations at diagnosis. Of these, 59% (10) displayed normal karyotype by conventional G-banding karyotyping, while hyperploidy with complex karyotypes was observed in only three cases. Fluorescence in situ hybridization (FISH) analysis was undertaken to detect *IGH* (14q32) translocation, *TP-53* deletion (17p) and 13q deletion in 6, 7 and 8 patients, respectively. None of these cases showed the presence of the tested abnormalities.

The mean duration of follow-up was three years (SD ± 2.5 years), and 39 deaths were recorded in the entire cohort. At least one relapse during the study period was reported in 79% (n = 87/110) of the participants, while 20 patients received stem cell transplants. Distribution of positive expression of markers in tumors is shown in Fig. 2.

Association of Clinicopathological Parameters with Survival

Amongst clinicopathological parameters, a significant association was observed between age groups and OS (p-value < 0.01). Patients were categorized into three age groups, < 50 years, 50–64 years and > 65 years. Survival analysis showed a mean survival time of 8.27 years (95% CI 6.38–10.16), 6.56 years (95% CI 5.15–7.97), and 3.76 years (95% CI 2.63–4.88) in the three age groups respectively (p-value < 0.01). The univariate Cox proportional hazard analysis revealed that advanced age is associated with a threefold increased risk of mortality (HR = 3.70, 95% CI-1.36-10.10) (see Fig. 3A and Supplementary Table 2).

Expression of Cell Markers and their association with Clinicopathological Parameters and Survival

Association between the expression of markers and clinicopathological variables was analyzed using the Chi2 test, and corresponding *p-values* were obtained in pairwise statistical analysis. The prognostic value of biomarkers was tested using the Log-rank test and reported with a 95% confidence interval using the Kaplan-Meir curve.

None of the tumors showed CD34 expression, while CD20 was expressed in 3.97% of the cases. Hence, CD34 and CD20 were excluded from further statistical analysis.

3.1.1 ALDH1

Cytoplasmic expression of ALDH1 was observed in 50% of the cases. However, no difference was found in overall survival (OS) when patients were stratified based on ALDH1 expression in tumors (ALDH1+ (mean survival 5.42 years, HR = 0.82, 95% CI-0.43-1.54)) (p-value > 0.05) (Fig. 3B) (Table 2).

Table 2
Univariate and multivariable analysis for association of clinicopathological and demographic factors, including expression of biomarkers by overall survival (OS) or hazard of mortality among patients with MM (n = 128)

Prognostic Factors	Crude Hazard Ratio (HR) (95% CI)	*p-value*	Adjusted Hazard Ratio (AHR) (95% CI)
Gender		0.52	
Female	1		
Male	1.25 (0.62–2.53)		
Age at Diagnosis		< 0.001	
< 50	1		1
50–64	1.56 (0.57–4.25)		1.59 (0.58–4.35)
> 65	3.70 (1.36–10.10)		4.10 (1.49–11.27)
ALDH1		**0.54**	
Negative	1		
Positive	0.82 (0.43–1.54)		
CD117		0.66	
Negative	1		
Positive	0.87 (0.46–1.63)		
CD45		0.81	
Negative	1		
Positive	1.11 (0.43–2.82)		
CD56		0.25	
Negative	1		
Positive	0.68 (0.36–1.30)		
MUC1		0.22	
Negative	1		
Positive	1.54 (0.76–3.13)		1.77 (0.85–3.60)
XBP1		0.95	
Negative	1		
Positive	1.01 (0.53–1.91)		1.06 (0.54–2.05)

A significant association was identified between patients diagnosed at an advanced age (> 57 years of age) and ALDH1 expression. A majority (65%) of the tumors from elderly patients expressed ALDH1 (p-value < 0.05). Significant associations were also identified between concordant expressions of ALDH1 with CD117 (p-value < 0.01) and CD56 (p-value < 0.01), however, these associations did not yield prognostic information in terms of OS or clinicopathological variables.

3.1.2 CD117

Membrane expression of CD117 was found in 48% of the cases and was associated with a 23% reduced risk of mortality (mean survival 6 years, HR = 0.87, 95% CI- 0.46–1.63) (p-value > 0.05). (Fig. 3C) (Table 2). Expression of CD117 showed significant concordant associations with CD56 (p-value < 0.05), and ALDH1 (p-value < 0.001) expressing tumors. No significant differences were observed between tumors with and without CD117 expression concerning ISS stage, bone lesions, relapse and other clinicopathological variables (P > 0.05).

3.1.3 CD56

Membrane expression of CD56 was detected in 62% of the patients and was found to be associated with a 32% reduced risk of mortality (mean survival 6.5 years, HR = 0.68, 95% CI – 0.36–1.30) when compared to cases lacking CD56 expression (P > 0.05) (Fig. 3D) (Table 2). Significant concordance was observed in expression of CD56, CD117 (p-value < 0.05), XBP1(p-value < 0.05) and ALDH1 (p-value < 0.001). No significant differences were observed between tumors expressing CD56 and clinicopathological variables (P > 0.05).

3.1.4 XBP1

Cytoplasmic and nuclear expression of XBP1 was observed in 50% of the cases. Significant associations were identified between concordant expressions of XBP1 with CD 117 (p-value < 0.001) and CD56 (p-value < 0.05) expression. We did not find any associations of XBP1 expression with patients' survival and clinicopathological variables (p-value > 0.05) (Fig. 3E) (Table 2).

3.1.5 MUC1

Sixty-five percent of the cases showed membranous and cytoplasmic expression of MUC1 in tumors. Cases stratified based on MUC1 expression did not show any associations with clinicopathological parameters and expression of other biomarkers. However, a survival advantage of 1.9 years was found to be associated with cases lacking MUC1 expression in their tumors (MUC1 + tumors; mean survival 5.15 years, MUC1- tumors; mean survival 7.05 years, HR = 1.54, 95% CI – 0.76–3.13)) (p-value > 0.05) (Fig. 3F) (Table 2).

Association of Combinatorial Groups with Survival

Combinatorial groups were analyzed to explore further the prognostic value of concordant expression of markers using the Chi^2 test.

3.1.6 ALDH1 & CD56; Combinatorial Group

Cases were categorized into four groups based on ALDH1 and CD56 expression. OS analysis delineated these combinations into four prognostic groups.

- *Good Prognosis: ALDH1-/CD56+, mean survival 6.57 ± 0.96 years (*n = 32)
- *Intermediate Prognosis ALDH1-/CD56-, mean survival 6.17 ± 0.96 years* (n = 32)
- *Poor Prognosis: ALDH1+/CD56+, mean survival 5.72 ± 0.41 years*, (n = 32)
- *Worst Prognosis: ALDH1+/CD56-, mean survival 3.89 ± 0.76 years* (n = 17)

Combinatorial group analysis corroborated the protective effect of CD56 expression, which was further enhanced in tumors lacking ALDH1 expression (*ALDH1-/CD56+)* (*mean survival 6.57 ± 0.96 years)*. Patients with tumors expressing ALDH1 but lacking CD56 expression (*ALDH1+/CD56-)* had the worst prognosis, with a mean OS of 3.89 years. In comparison, the expression of CD56 (*ALDH1+/CD56+)* increased the mean OS to 5.72 years (p-value > 0.05) (Fig. 4A).

3.1.7 XBP1 & CD117; Combinatorial Group

Combinatorial group analysis with XBP1 and CD117 was categorized into four prognostic groups as follows:

- *Good Prognosis: XBP1-/CD117-, mean survival 6.78 ± 0.81 years (*n = 41)
- *Intermediate Prognosis: XBP1-/CD117+, mean survival 5.87 ± 1.17 years (*n = 23)
- *Poor Prognosis: XBP1+/CD117+, mean survival 5.54 ± 0.46 years (*n = 39)
- *Worst Prognosis: XBP1+/CD117-, mean survival 4.14 ± 0.61 years (*n = 25)

Subgroup analysis of XBP1 and CD117 revealed the favorable prognostic effect of CD117 expression and adverse prognostic effect associated with XBP1 expression. Amongst cases lacking CD117 expression, patients with XBP1 expressing tumors showed 2.64 years reduced survival (*XBP1+/CD117-, mean survival 4.14 ± 0.61 years)*compared to patients with XBP1 negative tumors (*XBP1-/CD117-, mean survival 6.78 ± 0.81 years)* (p-value > 0.05) (Fig. 4B).

3.1.8 MUC1 & XBP1; Combinatorial Group

Independent expression of MUC1 and XBP1 showed a trend towards inferior OS. The prognostic value of the concordant expression of MUC1 and XBP1 was investigated in combinatorial groups. Subgroup analysis delineated into four distinct groups:

- *Good prognosis (MUC1-/XBP1-) mean survival of 8.02 ± 0.99 years (*n = 26)
- *Intermediate prognosis (MUC1- / XBP1+/) mean survival of 5.66 ± 0.66 years (*n = 19)
- *Poor prognosis (MUC1+ / XBP1-) mean survival of 5.14 ± 0.60 years (*n = 38)
- *Worst prognosis (MUC1+/ XBP1+) mean survival time of 4.6 ± 0.431 years (*n = 45)

Kaplan Meier's analysis revealed that concordant expression of MUC1 and XBP1 increased the risk of mortality. Though independent expression of either MUC1 or XBP1 [(XBP1+/ MUC1-) & (XBP1- / MUC1+)] showed increased survival advantage, however, 3.42 years of reduced survival was observed in patients whose tumors expressed both (XBP1+ / MUC1+). Only 50% of the patients with XBP1+/MUC1 + tumors survived for four years compared to 70% of patients surviving > 10 years when tumors lacked concordant expression of XBP1 and MUC1 (p-value 0.67) (Fig. 4C).

Multivariable Analysis Using Multiple Cox Regression Model for OS

We undertook multivariable analysis to examine the adjusted hazard ratios (AHR) associated with different variables. XBP1 was included in the multivariable analysis to evaluate its biological relevance in the presence of MUC1. We found poor prognosis associated with advanced age at the time of diagnosis (> 65 years) (adjusted HR = 4.10, 95% CI- 1.49–11.27) (p-value < 0.001) and the risk of mortality increased up to four folds in patients whose tumors expressed MUC1 (adjusted HR = 1.77, 95% CI-0.85- 3.60) (p-value 0.11) and XBP1 (adjusted HR = 1.06, 95% CI- 0.53–2.05) (p-value < 0.98) (Table 2).

4 Discussion

To our knowledge, this is the first study from Pakistan that describes the expression and prognostic significance of stem cell markers, lineage markers, MUC1, and XBP1 in BM trephines from patients with MM. The salient findings from this study are as follows:

1. MM patients in Pakistan present the disease approximately a decade earlier than in Western countries.
2. A high percentage of patients had tumors expressing stem cell-associated markers (CD117 and ALDH1) compared to the data from the rest of the world (see Table 3)
3. Combinatorial group analysis revealed the adverse prognostic value associated with ALDH1, XBP1, MUC1 and their concordant expression.

Table 3
Expression of biomarkers (%) in the studied cohort and their comparison with published studies

Marker	Expression Frequency in Tumors		
	Current Study (%)	Rest of the world (%)	Reference
CD34	0	0	(Kimlinger & Witzig, 1997)
ALDH1	51	19–30	(Ginestier et al., 2007)
CD117	48	32	(Mateo et al., 2008; Pan et al., 2016)
CD20	4	6–49	(Robillard et al., 2003; Mateo et al., 2008; Yavasoglu et al., 2015; Pan et al., 2016)
CD45	10.6	27, 73	(Santra, Shaughnessy Jr, & Bellamy, 2011; Flores-Montero et al., 2016)
CD56	60.3	60–74	(Guo et al., 2016; Pan et al., 2016)
MUC1	62.3	55–60	(Baldus, Palmen, & Thiele, 2007; Andrulis et al., 2014)
XBP1	48.3	70	(Carrasco et al., 2007)

MM patients presented a decade earlier in Pakistan

MM is considered a rare malignancy diagnosed between 65-and 74 years with a male preponderance [5, 28, 29]. In our study, the patients were diagnosed at least a decade earlier (median age 57, range 30–89 years) compared to 70 years in the western countries [30]. Studies from Pakistan have previously reported Early-onset of MM [31–35] and other Asian countries, including India [36, 37], Bangladesh [38], and China [39]. However, the differences between average life expectancies should be taken into consideration while comparing this data.

Kim, K et al. studied and compared the clinical features of MM patients in the Asian population between the 1960s and 2010 and discovered a link between population life expectancy and the median age at MM diagnosis. In the Korean population, they found that an increase in the average life expectancy from 64 years in 1960 to 81 years in 2010; induced an increase in the median age of MM diagnosis from 54 to 67 years during the same time points. They suggested exposure to chemical carcinogens, ionizing radiation, air pollution, Westernized diets, and obesity as contributing factors to a high MM incidence in their study population [40].

The average life expectancy in Pakistan is ~ 66 years compared to ~ 73 and 81 years in the US and UK, respectively [41]. If our population's life expectancy is extended to 81 years, it will be interesting to observe whether the finding by Kim et al. accurately depicts the projected connection between life expectancy and early diagnosis of MM in the Pakistani population. If the trend persists, it may prompt researchers to investigate the link between the early onset of MM and modifiable risk factors for MM.

ALDH1 is associated with an adverse prognosis

Aldehyde dehydrogenases (ALDHs) are a group of nicotinamide-adenine dinucleotide phosphate–positive (NAD(P)+) -dependent enzymes involved in retinoic acid metabolism and detoxification in cells. ALDHs govern drug resistance and differentiation in cancer stem cells [42, 43]. ALDH1 expression has been used to identify CSC-like populations in epithelial and hematological malignancies, including MM, and an association with drug resistance and relapse has been reported [44, 45]. Our study cohort showed ALDH1 expression in 50% of the tumors, higher than reported in other parts of the world. High expression of ALDH1 indicates the possible increase of CSC-like cells with increased resistance to therapy and undifferentiated phenotype. We could not explore the relationship between ALDH1 expression and increased drug resistance due to incomplete follow-up and variability in treatment regimens. However, immature morphological features were observed in CD138 expressing plasma cells. Low (4%) expression of CD20 in these cases further corroborates the finding.

The univariable analysis did not reveal any associations between ALDH1 and OS. However, combinatorial group analysis demonstrated adverse outcomes associated with ALDH1 expression in tumors lacking CD56 expression. Amongst patients with CD56 negative tumors, ALDH1 expression in tumors (ALDH1+/CD56-) showed 2.28 years of inferior survival, suggesting that CD56 expression abrogates the adverse effect of ALDH1.

CD56 expression and prognosis

CD56 is a neural cell adhesion molecule (NCAM) and a member of the immunoglobulin superfamily, expressed in developing and mature cells of the human nervous system and natural killer (NK) cells. It is expressed in 70–80% of cases of MM but not in normal plasma cells [46]. Our cohort showed CD56 expression in 60% of the cases, comparable to previous reports (see Table 3).

Koumpis et al. recently found that lack of CD56 expression in MM patients is associated with adverse prognostic value in terms of clinical presentation, such as elevated levels of lactate dehydrogenase (LDH) and β2-microglobulin levels. However, it did not affect patient survival [47]. Although we did not find any correlations between the expression of CD56 and patient survival, 70% of the cases diagnosed in advanced age (> 65 years) had CD56 expressing tumors (p-value < 0.05).

Combinatorial group analysis showed that CD56 expression abrogated the adverse prognostic value of ALDH1 expression. Amongst patients with ALDH1 expressing tumors, expression of CD56 contributed to a survival advantage of 1.83 years (mean survival 5.72 years).

Recently, Taouk and colleagues have demonstrated the potential of CD56 as a predictive marker for NK-mediated cytotoxicity in breast carcinoma cell lines. CD56 facilitated NK-mediated cytotoxicity by enhancing the interaction between NK and malignant cells, leading to cytotoxic enzyme transfer for NK cells. This transfer of cytotoxic enzyme induced activation of the Caspase-3 mediated activation in the target cell [48]. It is conceivable that expression of CD56 in myeloma cells induced activation of the NK mediated cytotoxic immune response, leading to cancer cell death, thereby contributing to favorable prognosis in patients.

The expression of CD56 also showed a significant association with CD117, which is another marker of CSC-like cells. The combinatorial group analysis corroborated the favorable outcome associated with CD56 expression without CD117.

CD117 expression and prognosis

CD117, commonly known as c-kit or stem cell factor receptor, is "KIT-proto-oncogene receptor tyrosine kinases" expressed on nonmalignant cells, including hematopoietic stem cells (HSCs), melanocytes, mast cells and breast epithelium, but not on plasma cells and in tumors such as on gastrointestinal stromal tumors, acute leukemias, myelodysplastic syndromes, monoclonal gammopathies and MM. CD117 plays a vital role in cell signaling, cell survival, proliferation, migration and cellular differentiation [49, 50]. No consensus has been established regarding the prognostic significance of CD117 expression in MM patients [3, 51].

In this study, CD117 was expressed in 48% of our cases compared to 32% reported in the literature. Increased expression of CD117 was anticipated, considering the high expression of ALDH1 and undifferentiated phenotype, as discussed earlier. Expression of CD117 showed significant association with ALDH1 (p-value 0.005), CD56 (p-value 0.01), and XBP1 (p-value 0.005) expression. When examined for associations with five-year survival, CD117 expression did not correlate with prognosis independently or in combinatorial analysis with ALDH1, CD56, and XBP1.

XBP1 expression and prognosis

XBP1 signaling pathway plays a vital role in the pathogenesis of MM. XBP1 is an essential mediator of the unfolded protein response (UPR) and a key component in the development of fetal hepatocytes, acinar cells, and plasma cells [52]. It acts as a multifunctional transcription factor (TF). There is increasing evidence regarding the role of XBP1 in cancers pathogenesis and its utility as a therapeutic target. *In vitro* studies have shown that overexpression of XBP1 increases proliferation and drug resistance in MM [20, 21, 53]. The pathogenic role of XBP1 in myeloma was best described by Carrasco et al. (2007) when they induced XBP1 expression in transgenic mice. All the mice developed MGUS with a resemblance to human disease. Also, within two years, 26% of the mice developed MM with increased secretion of monoclonal immunoglobulins [22]. Expression of XBP1 has been associated with adverse outcomes in BCa [54], B-cell lymphomas [55], and osteosarcomas [56]. However, the prognostic significance of XBP1 in MM is bipartisan. Bagratuni et al. have demonstrated that a high XBP1 spliced / unspliced ratio of mRNA is an independent prognostic indicator associated with poor survival in patients treated with thalidomide [21]. While Gambella et al. have shown that high expression of XBP1 mRNA is related to improved overall survival in patients treated with bortezomib [57]. Lee and colleagues have demonstrated that inhibiting the IRE1-XBP1 pathway in MM cells disrupts the activation of the spliced form of XBP1 and stabilizes the unspliced form acting as a double negative, thereby sensitizing the cells to ER stress-induced apoptosis [53].

In this study, the independent expression of XBP1 did not show any associations with clinicopathological parameters. Lack of XBP1 expression in tumors showed a survival advantage of 1.46 years compared to XBP1 + tumors. However, it did not reach statistical significance. The adverse prognostic value associated with XBP1 expression was further substantiated through XBP1 / CD117 combinatorial group analysis and a survival advantage of 2.64 years was found to be associated with a lack of XBP1 expression in CD117 negative tumors.

MUC1 expression and prognosis

MUC1 is a multifaceted protein playing a seminal role in tumorigenesis and its expression has been reported to be associated with poor overall and disease-free survival in carcinomas of the breast, prostate, lung, stomach, and ovary [58–60]. Hematopoietic cells express MUC1 to a lesser extent; however, its aberrant expression has been reported in hematological malignancies. *In vitro* studies in MM have demonstrated that expression of MUC1 is associated with CSC-like phenotype, drug resistance, and proliferation [11–13, 16, 17, 61]. This would suggest that *in vivo* expression of MUC1 may translate to an inverse outcome. Recently, the adverse prognostic effect of MUC1 expression has been reported in acute myeloid leukemia (AML) [62]. However, its prognostic relevance has not been reported in patients with MM. The reason studies do not demonstrate this adequately is due in part to the limited repertoire of antibodies to epitopes on different regions of MUC1: glycosylated, partially glycosylated, unglycosylated extracellular domain and to epitopes on the highly conserved cytoplasmic domain.

MUC1 is considered an attractive target for therapeutic intervention due to its differential expression patterns and glycosylation on normal and malignant cells. However, this variability in expression and glycosylation patterns contributes to inconsistencies in detecting MUC1 in diverse cancer types and within different cases of the same disease. Factors that should be taken into account while studying MUC1 are briefly described below:

1. Different components of MUC1 protein exhibit differential oncogenic activities. Expression of the secreted form of MUC1, also known as soluble MUC1 (sMUC1), is associated with tumor burden and can be effectively targeted using the B27.29 antibody for CA27.29 epitope present on sMUC1 [63]. Likewise, the MUC1 Ab-5 antibody detects the epitope present at the cytoplasmic tail (CT) at the carboxylic end of the protein. It provides significant information regarding the signaling functions of the protein and its association with stem cells [12]. Hence the expression of B27.29 may not yield information regarding the signaling molecules and MUC1 Ab-5 may not render any association with patient survival.
2. The Extracellular domain (ECD) of MUC1 contains VNTR, which consists of variable repeats of 20 aa. Five-potential sites within the VNTR domain are heavily glycosylated in healthy tissues, whereas overexpression of MUC1 during neoplastic transformation alters the glycosylation pattern of these sites and the tumor cells may express aberrantly glycosylated MUC1. The sugar moieties have been involved in antibody recognition and binding of the MUC1 antibodies developed against the epitopes

present in the VNTR domain [64, 65]. Therefore, patterns and degree of glycosylation may affect antibody-based detection of the protein

3. High polymorphism of MUC1 protein is attributed to the VNTR domain. It has been shown that variable amino acid sequences can exist in different cell lines with varying numbers of repeats. Furthermore, increased length of the VNTR domain may also result in poor antibody penetration and unavailability of the immunogen [66, 67]. Hence depending upon the various factors discussed above, studies evaluating the prognostic significance of MUC1 are inconclusive and partial.

In this study, a trend toward adverse outcomes was found to be associated with MUC1 expression. Patients with tumors lacking MUC1 expression showed a survival advantage of 2 years compared to MUC1 expressing tumors. Concordant expression of MUC1 with XBP1 further corroborated the adverse outcome observed with MUC1 expression. We found that in a small cohort of tumors negative for XBP1 expression, lack of MUC1 expression contributed to a survival benefit of almost 3-years with a mean survival of 8.02 years compared to a mean survival of 5.14 years in cases with MUC1expressing tumors. Concordant expression of MUC1 and XBP1 exerted a modifier effect on adverse outcomes with a means survival of 4.6 years. The effect raised a query about whether MUC1 and XBP1 interact at the molecular level leading to an adverse prognosis. Multivariable cox regression analysis showed that the expression of MUC1 and XBP1 in elderly patients (> 65 years) elevated the risk of mortality to 4-times compared to other age groups.

Provided that the findings related to the concordant expression of MUC1 and XBP1 are confirmed through studying larger cohorts, patients can be stratified further based on MUC1 and XBP1 expression at disease presentation. We can categorize the patient's prognosis and it may also facilitate the choice of treatment. Having observed the association between MUC1 and XBP1 and its inverse association with the outcome is intriguing and posits a question of potential functional relationship or cross-talk between them.

Proteins rarely act as isolated molecules in system biology and over 80% of the known proteins operate in complexes [68]. Furthermore, proteins undertaking similar molecular and cellular processes have been identified to be interacting with each other. The nature of this interaction and its downstream or upstream effects require further investigation. Likewise, considering the role of MUC1 and XBP1 in tumorigenesis, drug resistance, aberrant expression in tumors, and their associations with CSCs, it is conceivable that they interact at the molecular level, impacting cellular growth, migration, and invasion in MM. However, the hypothesis warrants further investigation.

A higher prevalence (11%) of Non-secretory Multiple Myeloma (NSMM) was identified in this study which was similar to a recent report from China, where 10.58% of non-secretary MM cases were reported [69]. NSMM has been reported as a rare variant representing 3–5% of MM patients. Recently, NSMM has been redefined and subclassified into four categories, a) oligo-secretors, b) non-producers, c) true non-secretors and d) false non-secretors [70, 71]. It is conceivable that the reanalysis of our cases with higher sensitive techniques and re-categorizing them according to the new criteria of NSMM may lower the prevalence of true NSMM in this study.

Furthermore, it is intriguing to speculate that this may be due to infection with Epstein Barr Virus (EBV), which was found in 7% of the tumors in a previous study by our group [27]. Host shutoff is one of the several host immune evasive approaches acquired by EBV where *BGLF5 (*an early lytic gene) affects generalized protein biosynthesis in host cells through mRNA degradation [72]. Further studies are required to determine the phenomenon of host shutoff in MM cells and their functional significance.

This is a single-center retrospective study. The minor variations in the results can be attributed to a) fixation protocols, b) antibodies used, c) scoring methods and d) sample size. Challenges encountered during our study were incomplete follow-up and a relatively small sample size. Additionally, our study cohort was a mixed group concerning treatment regimens since treatment strategies were not uniform. Including patients who received uniform treatment would further elaborate the association of these markers with the survival of patients in different treatment groups.

Declarations

7 Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by Ethics Review Committee, Aga Khan University, Karachi (2741-Pat-ERC-13). The patients included in the study provided written informed consent for the use of their tumor tissues and data for research.

8 Consent for publication

All the authors gave consent for the publication of the submitted article with details, including images and other details within the text to be published in *BMC* Cancer.

9 Availability of data

The datasets generated for this study are available on request to the corresponding author.

10 Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

11 Funding

The project was funded by the Aga Khan University (AKU) and generous donations.

12 Author’s contributions

ENL conceived the project. ENL and SR designed the experiments, reviewed the data and drafted the manuscript. SR conducted the experiments, undertook statistical analysis and interpretation of the data.

ENL, KM and SR undertook pathological review of the slides with interpretation. IA assisted with the statistical analysis. US facilitated the availability of samples and patient's data. All authors contributed to the article and approved the submitted version.

13 Acknowledgments

We acknowledge the contributions of Sadia Habib for technical assistance, and the Aga Khan University and the generosity of several donors for funding the project

References

1. Rajkumar SV: **Multiple myeloma: 2020 update on diagnosis, risk-stratification and management**. American journal of hematology 2020, **95**(5):548–567.
2. Schavgoulidze A, Cazaubiel T, Perrot A, Avet-Loiseau H, Corre J: **Multiple Myeloma: Heterogeneous in Every Way**. Cancers (Basel) 2021, **13**(6).
3. Mateo G, Montalban MA, Vidriales MB, Lahuerta JJ, Mateos MV, Gutierrez N, Rosinol L, Montejano L, Blade J, Martinez R *et al*: **Prognostic value of immunophenotyping in multiple myeloma: a study by the PETHEMA/GEM cooperative study groups on patients uniformly treated with high-dose therapy**. J Clin Oncol 2008, **26**(16):2737–2744.
4. Joao C, Costa C, Coelho I, Vergueiro MJ, Ferreira M, da Silva MG: **Long-term survival in multiple myeloma**. Clin Case Rep 2014, **2**(5):173–179.
5. Fonseca R, Abouzaid S, Bonafede M, Cai Q, Parikh K, Cosler L, Richardson P: **Trends in overall survival and costs of multiple myeloma, 2000–2014**. Leukemia 2017, **31**(9):1915–1921.
6. Bonnet D, Dick JE: **Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell**. Nature medicine 1997, **3**:730.
7. Johnsen HE, Kjeldsen MK, Urup T, Fogd K, Pilgaard L, Boegsted M, Nyegaard M, Christiansen I, Bukh A, Dybkaer K: **Cancer stem cells and the cellular hierarchy in haematological malignancies**. Eur J Cancer 2009, **45 Suppl 1**:194–201.
8. Hawley RG: **The cancer stem cell conundrum in multiple myeloma**. 2012.
9. Drewinko B, Alexanian R, Boyer H, Barlogie B, Rubinow SI: **The growth fraction of human myeloma cells**. Blood 1981, **57**(2):333–338.
10. Brugger W, Buhring HJ, Grunebach F, Vogel W, Kaul S, Muller R, Brummendorf TH, Ziegler BL, Rappold I, Brossart P *et al*: **Expression of MUC-1 epitopes on normal bone marrow: implications for the detection of micrometastatic tumor cells**. J Clin Oncol 1999, **17**(5):1535–1544.
11. Yin L, Kosugi M, Kufe D: **Inhibition of the MUC1-C oncoprotein induces multiple myeloma cell death by down-regulating TIGAR expression and depleting NADPH**. Blood 2012, **119**(3):810–816.
12. Tagde A, Rajabi H, Bouillez A, Alam M, Gali R, Bailey S, Tai YT, Hideshima T, Anderson K, Avigan D *et al*: **MUC1-C drives MYC in multiple myeloma**. Blood 2016, **127**(21):2587–2597.

13. Rosenblatt J, Stroopinsky D, Mills H, Jakubikova J, Clohessy JG, Neeharika S, Vasir B, Rajabi H, Coll MD, Palmer K: **Co-Expression Of The MUC1 Oncoprotein and CD34 On Primary Myeloma Bone Marrow Cells Identifies a Population With Myeloma Initiating Potential**. In.: Am Soc Hematology; 2013.
14. Huang L, Chen D, Liu D, Yin L, Kharbanda S, Kufe D: **MUC1 oncoprotein blocks glycogen synthase kinase 3beta-mediated phosphorylation and degradation of beta-catenin**. Cancer Res 2005, **65**(22):10413–10422.
15. Ahmad R, Raina D, Trivedi V, Ren J, Rajabi H, Kharbanda S, Kufe D: **MUC1 oncoprotein activates the IkappaB kinase beta complex and constitutive NF-kappaB signalling**. Nat Cell Biol 2007, **9**(12):1419–1427.
16. Kawano T, Ahmad R, Nogi H, Agata N, Anderson K, Kufe D: **MUC1 oncoprotein promotes growth and survival of human multiple myeloma cells**. Int J Oncol 2008, **33**(1):153–159.
17. Bar-Natan M, Stroopinsky D, Luptakova K, Coll MD, Apel A, Rajabi H, Pyzer AR, Palmer K, Reagan MR, Nahas MR *et al*: **Bone marrow stroma protects myeloma cells from cytotoxic damage via induction of the oncoprotein MUC1**. *British journal of haematology* 2017, **176**(6):929–938.
18. Stroopinsky D, Kufe D, Avigan D: **MUC1 in hematological malignancies**. Leuk Lymphoma 2016, **57**(11):2489–2498.
19. Paydas S, Sahin B, Gonlusen G, Hazar B, Zorludemir S: **MUC1 expression in plasmacytoma**. Leuk Res 2001, **25**(3):221–225.
20. Nakamura M, Gotoh T, Okuno Y, Tatetsu H, Sonoki T, Uneda S, Mori M, Mitsuya H, Hata H: **Activation of the endoplasmic reticulum stress pathway is associated with survival of myeloma cells**. Leuk Lymphoma 2006, **47**(3):531–539.
21. Bagratuni T, Wu P, Gonzalez de Castro D, Davenport EL, Dickens NJ, Walker BA, Boyd K, Johnson DC, Gregory W, Morgan GJ *et al*: **XBP1s levels are implicated in the biology and outcome of myeloma mediating different clinical outcomes to thalidomide-based treatments**. Blood 2010, **116**(2):250–253.
22. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS: **The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis**. Cancer cell 2007, **11**(4):349–360.
23. Kurata M, Yamazaki Y, Kanno Y, Ishibashi S, Takahara T, Kitagawa M, Nakamura T: **Anti-apoptotic function of Xbp1 as an IL-3 signaling molecule in hematopoietic cells**. Cell death & disease 2011, **2**(2):e118.
24. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM: **Reporting Recommendations for Tumor Marker Prognostic Studies**. Journal of Clinical Oncology 2005, **23**(36):9067–9072.
25. **World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects**. Jama 2013, **310**(20):2191–2194.
26. Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG: **Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration**. Journal of

the National Cancer Institute 2018, **110**(8):803–811.

27. Rajput S, Minhas K, Azam I, Shaikh U, Hussain A, Lalani EN: **LMP1 expression in bone marrow trephines of patients with multiple myeloma confers a survival advantage**. Leuk Lymphoma 2019, **60**(8):1991–2001.
28. Becker N: **Epidemiology of multiple myeloma**. In: *Multiple Myeloma.* edn.: Springer; 2011: 25–35.
29. Noone AM HN, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds).: **SEER Cancer Statistics Review, 1975–2015,National Cancer Institute. Bethesda, MD, based on November 2017 SEER data submission, posted to the SEER website, April** 2018. **https://seer.cancer.gov/csr/1975_2015/**,. 2018.
30. Zhou L, Yu Q, Wei G, Wang L, Huang Y, Hu K, Hu Y, Huang H: **Measuring the global, regional, and national burden of multiple myeloma from 1990 to 2019**. BMC Cancer 2021, **21**(1):606.
31. Shaheen H, Ghanghroo I, Malik I: **Clinicopathological features and management of Pakistani patients with multiple myeloma**. J Pak Med Assoc 1999, **49**(10):233–237.
32. Sultan S, Irfan SM, Parveen S, Ali H, Basharat M: **Multiple Myeloma: a Retrospective Analysis of 61 Patients from a Tertiary Care Center**. Asian Pac J Cancer Prev 2016, **17**(4):1833–1835.
33. Raziq F, Tahir M, Wazir R, Rafiq A: **Hematological Presentation of Multiple Myeloma in Khyber Pakhtunkhwa**. Gomal Journal of Medical Sciences 2010, **8**(2).
34. Basit A, Siddiqui N, Hameed A, Muzaffar N, Athar S: **Factors affecting outcome of patients with multiple myeloma**. J Ayub Med Coll Abbottabad 2014, **26**(3):376–379.
35. Mansoor S, Siddiqui I, Adil S, Nabi Kakepoto G, Fatmi Z, Ghani F: **Anion gap among patients of multiple myeloma and normal individuals**. Clin Biochem 2007, **40**(3–4):226–229.
36. Konatam AML, Sadashivudu G: **Age of onset of multiple myeloma: a paradigm shift in Indian patients**. Indian J Appl Res 2016, **6**(10):3.
37. Yanamandra U, Khattry N, Kumar S, Raje N, Jain A, Jagannath S, Menon H, Kumar L, Varma N, Varma S: **Consensus in the management of multiple myeloma in India at myeloma state of the art** 2016 **conference**. *Indian Journal of Hematology and Blood Transfusion* 2017, **33**(1):15–21.
38. Hossain MS, Iqbal MS, Khan MA, Rabbani MG, Khatun H, Munira S, Miah MMZ, Kabir AL, Islam N, Dipta TF: **Diagnosed hematological malignancies in Bangladesh-a retrospective analysis of over 5000 cases from 10 specialized hospitals**. *BMC cancer* 2014, **14**(1):438.
39. Lu J, Chen W, Huo Y, Huang X, Hou J: **Clinical features and treatment outcome in newly diagnosed Chinese patients with multiple myeloma: results of a multicenter analysis**. Blood cancer journal 2014, **4**(8):e239.
40. Kim K, Lee JH, Kim JS, Min CK, Yoon SS, Shimizu K, Chou T, Kosugi H, Suzuki K, Chen W *et al*: **Clinical profiles of multiple myeloma in Asia-An Asian Myeloma Network study**. Am J Hematol 2014, **89**(7):751–756.
41. WHO: **Global Health Observatory data repository, Life expectancy and Healthy life expectancy Data by WHO region**. In.; 2019.

42. Guo W, Wang H, Chen P, Shen X, Zhang B, Liu J, Peng H, Xiao X: **Identification and Characterization of Multiple Myeloma Stem Cell-Like Cells**. Cancers (Basel) 2021, **13**(14).
43. Tomita H, Tanaka K, Tanaka T, Hara A: **Aldehyde dehydrogenase 1A1 in stem cells and cancer**. Oncotarget 2016, **7**(10):11018–11032.
44. Zhou W, Yang Y, Gu Z, Wang H, Xia J, Wu X, Zhan X, Levasseur D, Zhou Y, Janz S *et al*: **ALDH1 Activity Identifies Tumor Initiating Cells and Links to Chromosomal Instability Signatures in Multiple Myeloma**. Leukemia 2014, **28**(5):1155–1158.
45. Boucher K, Parquet N, Widen R, Shain K, Baz R, Alsina M, Koomen J, Anasetti C, Dalton W, Perez LE: **Stemness of B-cell progenitors in multiple myeloma bone marrow**. Clinical cancer research: an official journal of the American Association for Cancer Research 2012, **18**(22):6155–6168.
46. Khallaf SM, Yousof EA, Ahmed EH, Mansor SG, Mohamed HO, Elgammal S, Moeen SM, Sayed DM: **Prognostic Value of CD56 Expression in Multiple Myeloma**. Research in Oncology 2020, **16**(1):6–10.
47. Koumpis E, Tassi I, Malea T, Papathanasiou K, Papakonstantinou I, Serpanou A, Tsolas E, Kapsali E, Vassilakopoulos TP, Papoudou-Bai A *et al*: **CD56 expression in multiple myeloma: Correlation with poor prognostic markers but no effect on outcome**. Pathology - Research and Practice 2021, **225**:153567.
48. Taouk G, Hussein O, Zekak M, Abouelghar A, Al-Sarraj Y, Abdelalim EM, Karam M: **CD56 expression in breast cancer induces sensitivity to natural killer-mediated cytotoxicity by enhancing the formation of cytotoxic immunological synapse**. Scientific Reports 2019, **9**(1):8756.
49. Foster BM, Zaidi D, Young TR, Mobley ME, Kerr BA: **CD117/c-kit in Cancer Stem Cell-Mediated Progression and Therapeutic Resistance**. Biomedicines 2018, **6**(1):31.
50. Foster BM, Zaidi D, Young TR, Mobley ME, Kerr BA: **CD117/c-kit in Cancer Stem Cell-Mediated Progression and Therapeutic Resistance**. Biomedicines 2018, **6**(1).
51. Pan Y, Wang H, Tao Q, Zhang C, Yang D, Qin H, Xiong S, Tao L, Wu F, Zhang J: **Absence of both CD56 and CD117 expression on malignant plasma cells is related with a poor prognosis in patients with newly diagnosed multiple myeloma**. Leukemia research 2016, **40**:77–82.
52. Glimcher L: **XBP1: the last two decades**. Annals of the rheumatic diseases 2010, **69**(Suppl 1):i67-i71.
53. Lee A-H, Iwakoshi NN, Anderson KC, Glimcher LH: **Proteasome inhibitors disrupt the unfolded protein response in myeloma cells**. *Proceedings of the National Academy of Sciences* 2003, **100**(17):9946–9951.
54. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y: **XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway**. Nature 2014, **508**(7494):103.
55. Balague O, Mozos A, Martinez D, Hernandez L, Colomo L, Mate JL, Teruya-Feldstein J, Lin O, Campo E, Lopez-Guillermo A *et al*: **Activation of the endoplasmic reticulum stress-associated transcription factor x box-binding protein-1 occurs in a subset of normal germinal-center B cells and in aggressive B-cell lymphomas with prognostic implications**. The American journal of pathology 2009, **174**(6):2337–2346.

56. Sun Y, Jiang F, Pan Y, Chen X, Chen J, Wang Y, Zheng X, Zhang J: **XBP1 promotes tumor invasion and is associated with poor prognosis in oral squamous cell carcinoma**. Oncology reports 2018, **40**(2):988–998.
57. Gambella M, Rocci A, Passera R, Gay F, Omedè P, Crippa C, Corradini P, Romano A, Rossi D, Ladetto M *et al*: **High XBP1 expression is a marker of better outcome in multiple myeloma patients treated with bortezomib**. Haematologica 2014, **99**(2):e14-e16.
58. Nath S, Mukherjee P: **MUC1: a multifaceted oncoprotein with a key role in cancer progression**. Trends in molecular medicine 2014, **20**(6):332–342.
59. Jing X, Liang H, Hao C, Yang X, Cui X: **Overexpression of MUC1 predicts poor prognosis in patients with breast cancer**. Oncology reports 2019, **41**(2):801–810.
60. Xu F, Liu F, Zhao H, An G, Feng G: **Prognostic Significance of Mucin Antigen MUC1 in Various Human Epithelial Cancers: A Meta-Analysis**. Medicine (Baltimore) 2015, **94**(50):e2286.
61. Grant S: **Bortezomib resistance and MUC1 in myeloma**. Blood 2014, **123**(19):2910–2912.
62. Stroopinsky D, Rajabi H, Nahas M, Rosenblatt J, Rahimian M, Pyzer A, Tagde A, Kharbanda A, Jain S, Kufe T *et al*: **MUC1-C drives myeloid leukaemogenesis and resistance to treatment by a survivin-mediated mechanism**. *Journal of cellular and molecular medicine* 2018.
63. Treon SP, Maimonis P, Bua D, Young G, Raje N, Mollick J, Chauhan D, Tai YT, Hideshima T, Shima Y *et al*: **Elevated soluble MUC1 levels and decreased anti-MUC1 antibody levels in patients with multiple myeloma**. Blood 2000, **96**(9):3147–3153.
64. Movahedin M, Brooks TM, Supekar NT, Gokanapudi N, Boons G-J, Brooks CL: **Glycosylation of MUC1 influences the binding of a therapeutic antibody by altering the conformational equilibrium of the antigen**. Glycobiology 2017, **27**(7):677–687.
65. Taylor-Papadimitriou J, Burchell JM, Graham R, Beatson R: **Latest developments in MUC1 immunotherapy**. Biochemical Society Transactions 2018.
66. Singh R, Bandyopadhyay D: **MUC1: a target molecule for cancer therapy**. Cancer Biol Ther 2007, **6**(4):481–486.
67. Engelmann K, Baldus SE, Hanisch F-G: **Identification and topology of variant sequences within individual repeat domains of the human epithelial tumor mucin MUC1**. Journal of Biological Chemistry 2001, **276**(30):27764–27769.
68. Rao VS, Srinivas K, Sujini GN, Kumar GNS: **Protein-Protein Interaction Detection: Methods and Analysis**. International Journal of Proteomics 2014, **2014**:12.
69. Qin X-Q, An G, Li Z-J, Liu L-T, Xu Y, Yang L-H, Ma Y-P, Deng S-H, Sui W-W, Qin Y: **Secretory status of monoclonal immunoglobulin is related to the outcome of patients with myeloma: a retrospective study**. Blood advances 2019, **3**(5):751–760.
70. Corso A, Mangiacavalli S: **Non-Secretory Myeloma: Ready for a new Definition?** Mediterranean journal of hematology and infectious diseases 2017, **9**(1):e2017053-e2017053.

71. Dupuis MM, Tuchman SA: **Non-secretory multiple myeloma: from biology to clinical management**. OncoTargets and therapy 2016, **9**:7583–7590.

72. Rowe M, Glaunsinger B, van Leeuwen D, Zuo J, Sweetman D, Ganem D, Middeldorp J, Wiertz EJ, Ressing ME: **Host shutoff during productive Epstein–Barr virus infection is mediated by BGLF5 and may contribute to immune evasion**. *Proceedings of the National Academy of Sciences* 2007, **104**(9):3366–3371.

Figures

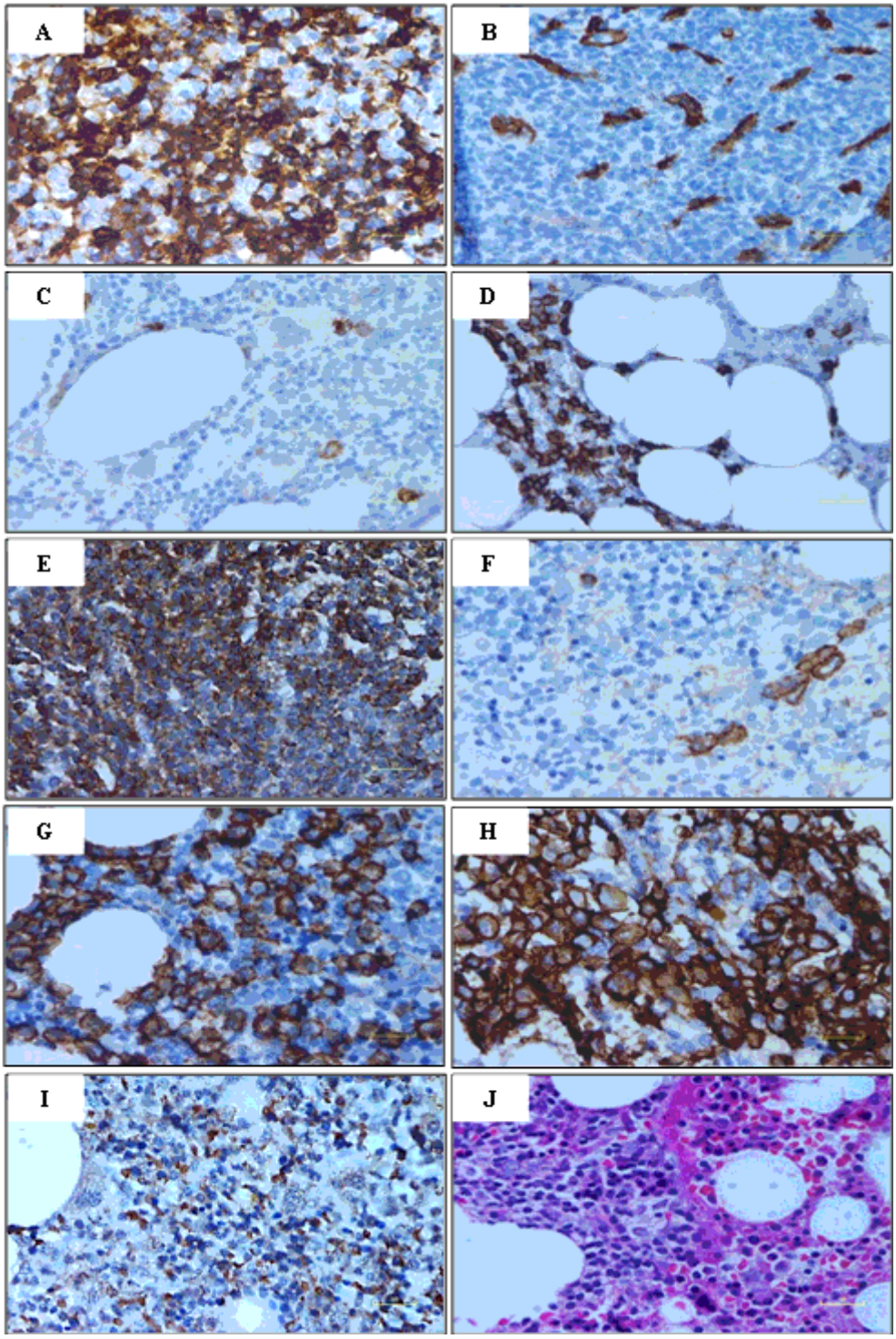

Figure 1

Photomicrographic collage showing expression of markers

(A) Membrane expression of ALDH1, (B) Scattered tumor cells and endothelial cells (white arrow) showing membranous CD34 expression, (C) Isolated cells showing membrane expression of CD117, (D) cluster of tumor cells showing CD20 membranous expression, (E) Diffuse membranous expression of CD45, (F) Membrane expression of CD56, (G) Membrane expression of CD138, (H) Membranous and cytoplasmic expression of MUC1(EMA),(I) Weak nuclear and cytoplasmic expression of XBP1, (J) hematoxylin and eosin staining of trephine biopsy.

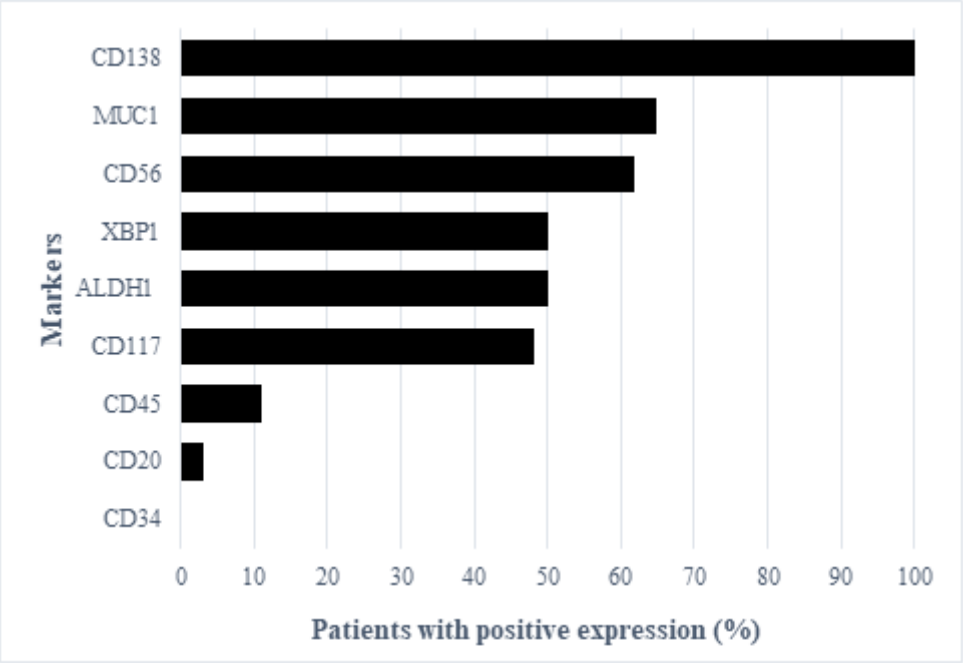

Figure 2

Distribution of positive expression of markers among MM patients

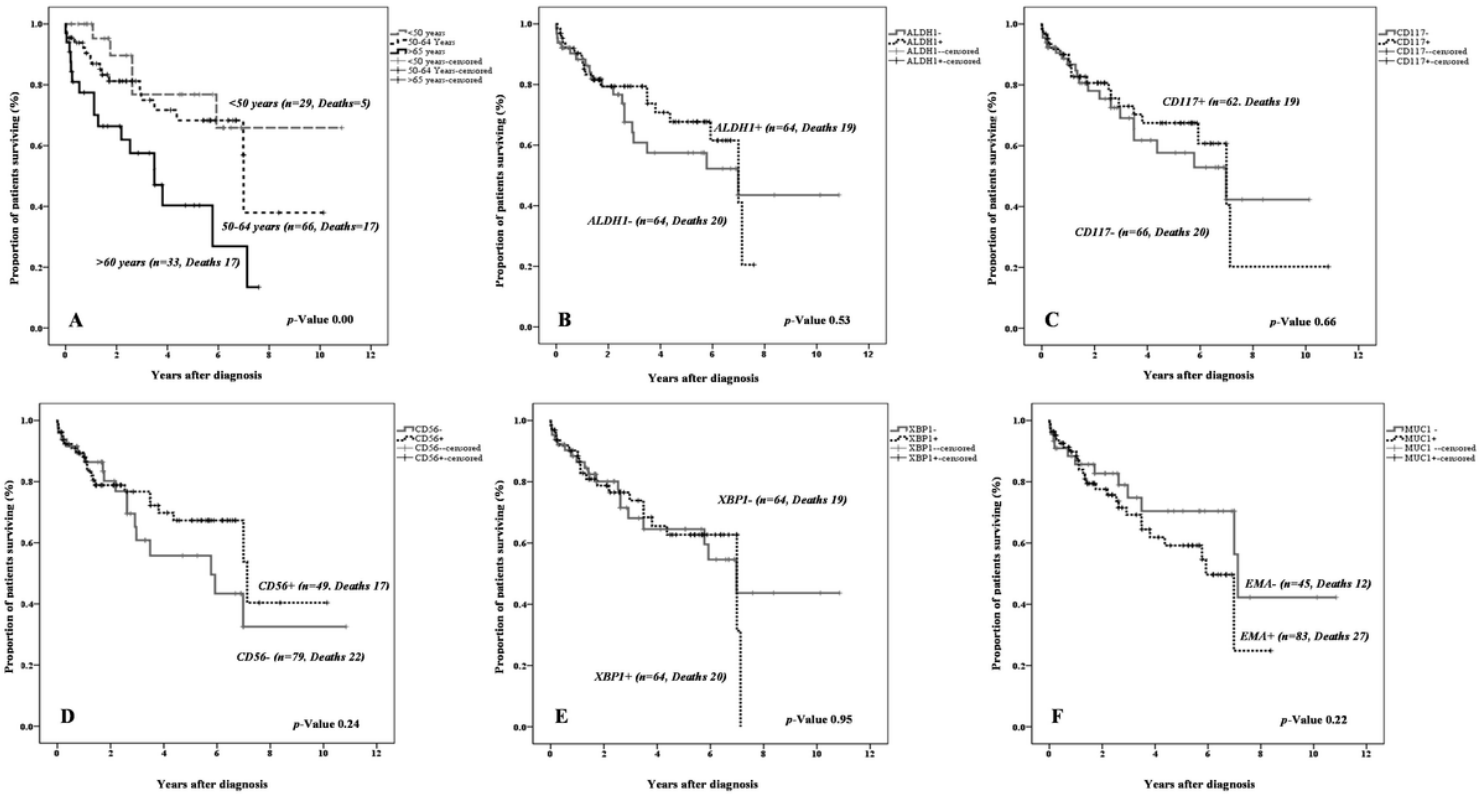

Figure 3

Kaplan Meier Curve- Five-year survival of patients by

(A) Age (p-value <0.001), (B) ALDH1(p-value 0.53), (C) CD117 (p-value 0.66), (D) CD56 (p-value 0.24), (E) XBP1 (p-value 0.95) and, (F) MUC1 (p-value 0.22).

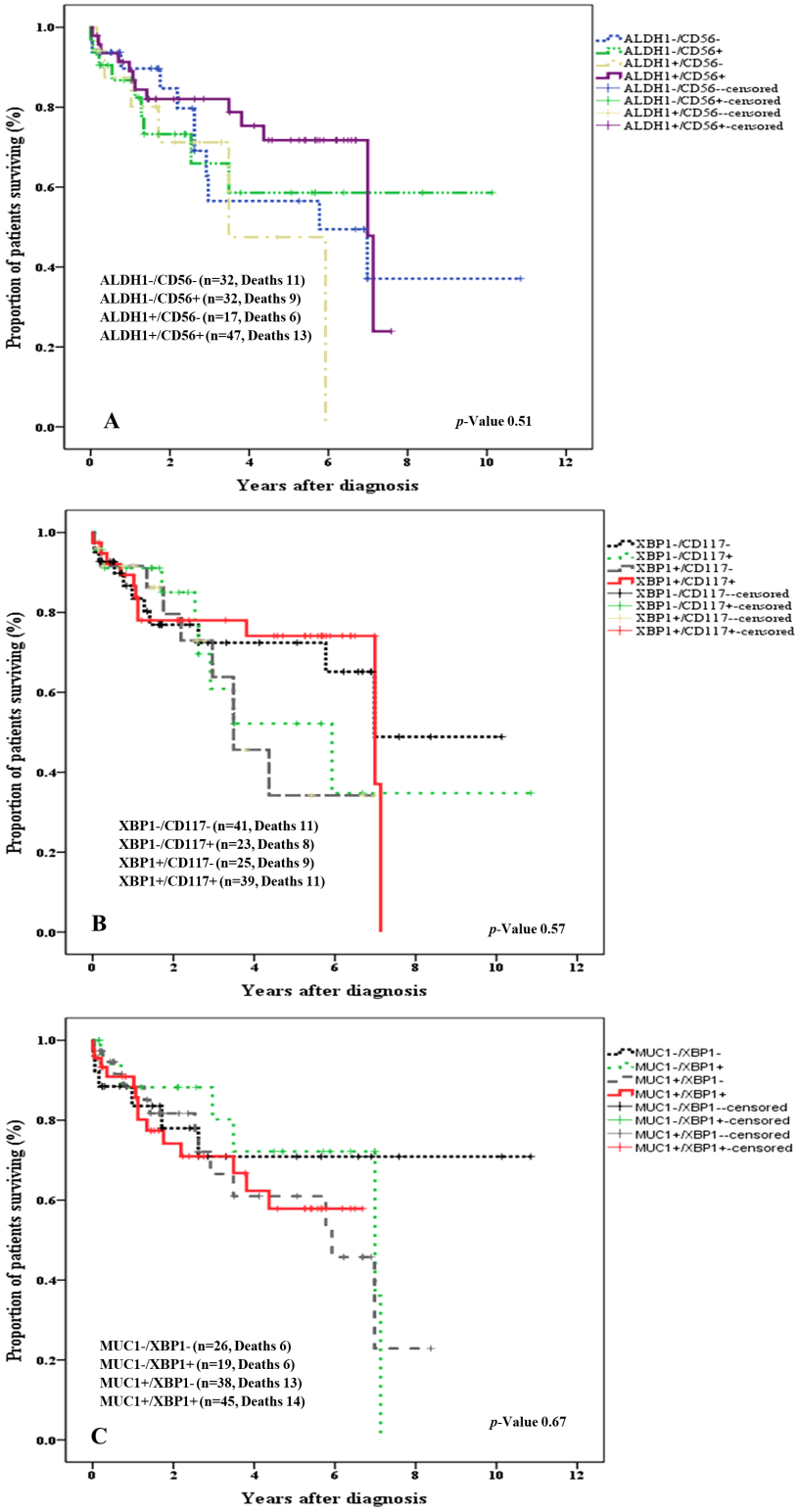

Figure 4

Kaplan Meier Curve showing OS for:

(A) combinatorial expression of ALDH1 and CD56, (B) combinatorial expression of XBP1 and CD117, and (C) Combinatorial expression of MUC1 and XBP1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.docx
- SupplementaryTable1.docx
- SupplementaryTable2.docx