Surveying surface antigens expression in multiple myeloma preclinical models

N. Morisot, J. Tam, N. Dailey, T. Davis, J. Chen, J. Islar, K. Pratt, N. Kimura, L. Buren, M. Whang, N. Patel, S. Lazetic, I. Chan, J.B. Trager, J.B.L. Tan

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Introduction

Multiple myeloma (MM) is a progressive hematological cancer with a 5-year survival rate of 53% (1). Novel therapeutic strategies are being developed to target specific MM surface antigens. Yet, changes in antigen expression through MM progression are poorly understood in the clinic and have not been well characterized in preclinical models.

Aim

Understand how the surface expression patterns of MM-associated antigens BCMA, CD38, CD138 and HLA-DR may be affected as MM progresses in preclinical models

BCMA and CD38 expression is frequently elevated in MM patient samples

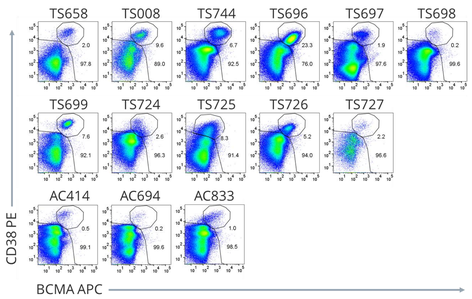


Figure 1: Flow cytometry analysis performed on cryopreserved bone marrow monocytes (BM MNC) from ten MM patients (Tissue Solutions, TS) and healthy control (AllCell, AC). Top right gate shows BCMA^{+}CD38^{hi} BM MNCs.

BCMA, CD38 and CD138 are highly expressed in commercially available MM cell lines

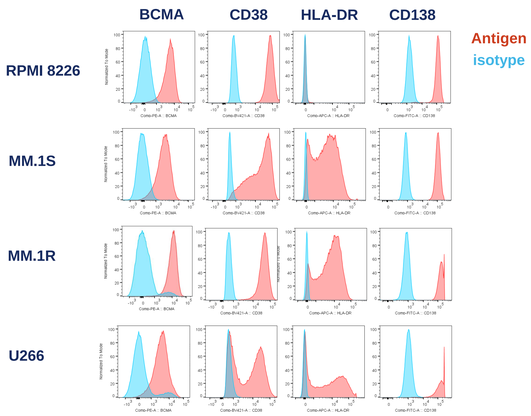


Figure 2: Flow cytometry analysis performed on MM cell lines RPMI 8266, MM.1S, MM.1R and U266 after <5 in vitro passages (P5)

Key features of MM cell lines

	RPMI-8226	MM.1S	MM.1R	U266
Donor Sex/Age	M/61	F/42		M/53
Microsatellites status	Stable	Instable (MSI-low)	ND	Stable
Canonical translocation	t(16;22) + t(8;22)	t(14:16) + t(8;14)		t(11;14)
Gene mutation				
CDKN2C	HD	HD		ND
KRAS	G12A - Het (cc)	G12A - Het (cc)		Wt
NRAS	Wt	Wt		ND
TP53	E285K - Homo (cc)	Wt		A161T - Homo (cc)
TRAF3	HD -> K191LfsX60	K536-N545delinsD - Homo (cc)		K550IfsX3 - Homo (cc)

https://www.keatslab.org/myeloma-cell-lines/hmcl-characteristics
ND=no data available

MM antigen levels are sensitive to extended culture and cell isolation methods

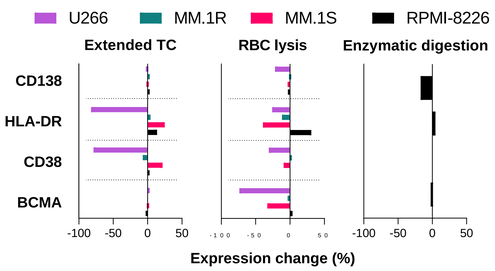


Figure 3: Antigen levels analyzed by flow cytometry and shown as % change from expression measured at P5 (Fig. 2). Extended tissue culture (TC) corresponds to P20. Enzymatic digestion tested on intended subcutaneous xenograft model only (RPMI-8226).

HLA-DR and CD138 levels in RPMI-8226 s.c. model are inconsistent with in vitro data

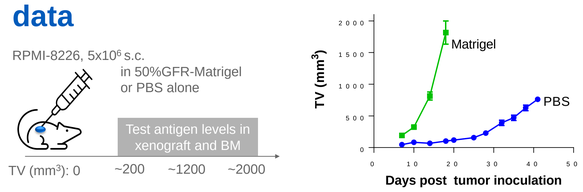


Figure 4: Left, RPMI-8226 was inoculated subcutaneously (s.c.) at 5x10^6 cells in 50% growth factor-reduced matrigel or PBS alone in NSG mice. Xenografts and bone marrow (BM) were harvested at various tumor volumes (TV) for flow cytometry analysis of antigens expression (Figure 5). Right: Xenograft TV over time.

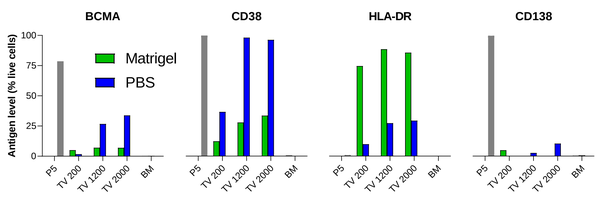


Figure 5: Flow cytometry analysis of antigen levels in xenografts at various TV and BM of RPMI-8226 tumor-bearing mice compared to in vitro levels (grey) measured at P5

Antigen detection in the bone marrow confirms engraftment of MM cells after i.v. injection

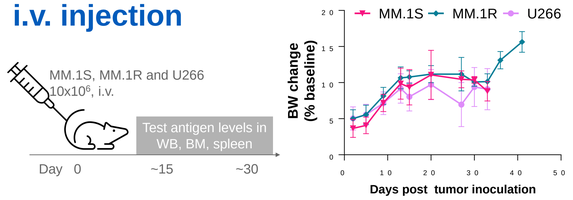


Figure 6: Left, MM cells were inoculated intravenously (i.v.) at 10x10^6 cells in PBS in NSG mice. Whole blood (WB), BM and spleen were harvested ~15 and ~30 days post-injection for analysis of antigens expression (Figure 7). Right, Body weight (BW) change from baseline over time. Baseline BW was measured one day before inoculation.

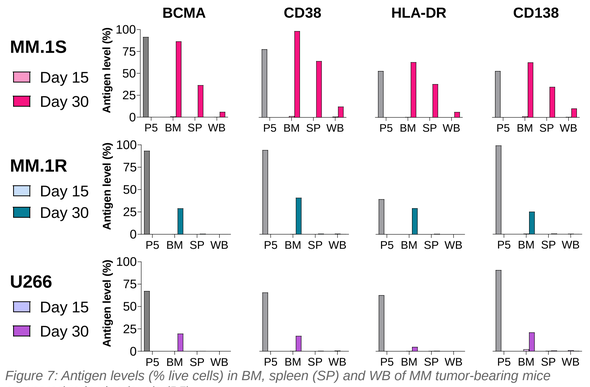


Figure 7: Antigen levels (% live cells) in BM, spleen (SP) and WB of MM tumor-bearing mice compared to in vitro levels (P5)

GFP/Luc-engineered MM.1S and MM.1R show high BCMA and CD38 levels

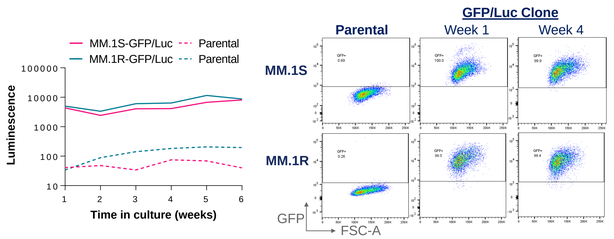


Figure 8: MM.1S and MM.1R parental cell lines were transfected with green fluorescent protein (GFP) and luciferase (Luc) reporter genes and maintained in culture for up to 6 weeks. Stable Luc signal (left, Bright-glo™ assay) and GFP expression (right, flow cytometry) in selected clones.

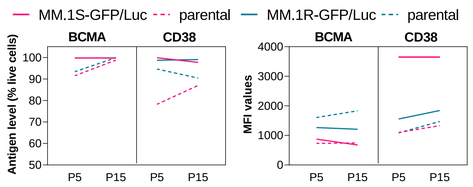
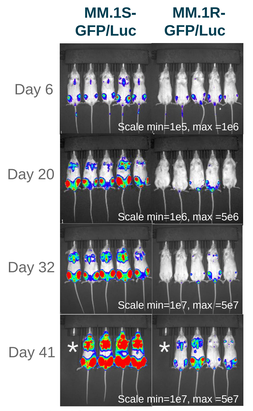


Figure 9: Antigen levels (Left) and MFI values (right) in MM.1S- and MM.1R-GFP/Luc compared to parental cell lines measured at P5 and P15. HLA-DR and CD138 were not tested

MM.1S-GFP/Luc is a more aggressive MM model compared to MM.1R-GFP/Luc



**Used for Day 35 ex vivo analysis*

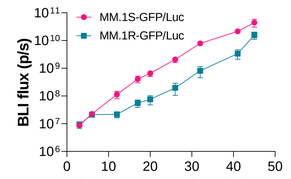


Figure 10: BLI images (Left) and flux (top) from NSG mice injected i.v. with selected MM.1S- and MM.1R-GFP/Luc at 10x10^6 cells/animal in PBS on Day 0

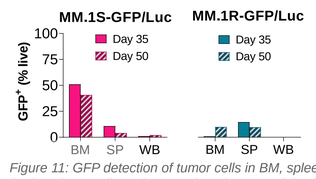


Figure 11: GFP detection of tumor cells in BM, spleen (SP) and WB collected ~35 and 50 days post-injection

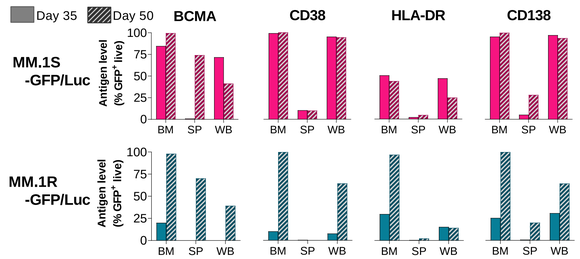


Figure 12: Antigen levels in ex vivo tissue from GFP/Luc-engineered MM tumor-bearing mice

Conclusion

Our data highlight antigen expression differences in MM cells when analyzed in mouse tissue compared to in vitro culture. Like the widely variable expression observed between patients (2, 3), BCMA and CD138 were differentially expressed in the mouse bone marrow between MM models. Commonly targeted antigens in MM also vary kinetically in vivo and can be measured and tracked using flow cytometry. The present findings also support the use of MM.1S cell lines when assessing BCMA, CD38 and CD138-specific immunotherapies or combinatorial approaches to MM treatment.

References

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Contact

James Trager, PhD
jtrager@nkartatx.com
www.nkartatx.com