Multiple Myeloma Cells Express Functional E-Selectin Ligands Which Can be Inhibited Both in vitro and in vivo Leading to Prolongation of Survival in a Murine Transplant Model

Alessandro Natoni, PhD,1,2,*, Michele Moschetta, MD,1,2,*, Siobhan Glavey, MD,1,2,3,*, Ping Wu, MBBS, MSc1,*, Gareth J. Morgan, MD, PhD,†, Lokesh Joshi, PhD1,†, John L. Magnani, PhD,‡, Irene M. Gobrial, MD,§ and Michael E O’Dwyer, MD, FRCPI, FRCPath1,2

1Glycoscience Research Group, National University of Ireland Galway, Ireland, 2Hematology, National University of Ireland Galway, Ireland, 3Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, 4Hematology-Oncology Research Unit, Division of Molecular Pathology, The Institute of Cancer Research, London, United Kingdom, 5The Royal Marsden Hospital, London, United Kingdom, 6GlycoMimetics, Inc., Gaithersburg, MD, 7Jerome Liperi Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA. *equal contribution

Multiple Myeloma

- Multiple Myeloma (MM) is an incurable disease and the second most common hematological malignancy
- MM resembles the long-lived, post germinal centre plasma cells but unlike normal plasma cells, MM cells retain the ability to proliferate
- A key feature of MM is the intimate connection and dependency of malignant cells with the bone marrow (BM) microenvironment

E-Selectin

- E-selectin regulates the Step-1 rolling interaction required for tissue-specific cell migration and is constitutively expressed in the specialized BM endothelium where it regulates the migration and recruitment of Human Stem/Progenitor cells (HSPCs) to the BM
- Functional E-Selectin ligands are characterised by reactivity with the Heca452 antibody
- Increased expression of ST3GAL6, an enzyme involved in E-selectin ligand synthesis, is independently associated with poor survival in MM

Objectives

- Assess the expression of E-Selectin Ligands on MM cell lines using the Heca452 antibody
- Determine the functionality of the E-Selectin Ligands under dynamic flow conditions using microfluidic chambers coated with recombinant E-Selectin
- Examine the importance of E-Selectin/E-Selectin ligand interaction in MM disease using a xenograft mouse model and a novel glycomimetic E-selectin inhibitor GMI1271

MM Cell lines display low positivity to Heca452

The indicated cell lines were stained for 30 min with the Heca452-PE antibody and then analysed by flow cytometry. Bars represent mean ±SD of five independent repeats

Hypoxia increases the percentage of Heca452 positive cells in RPMI8226

RPMI8226 Cells (5 Days)

% Heca452-PE Positive Cells

H929

MM1S

RPMI8226

Hypoxia (21% O2)

H929

MM1S

RPMI8226

Hypoxia (1% O2)

RPMI8226 cells were grown in hypoxic (1% O2) or normoxic (21% O2) conditions for five days and then stained with the Heca452-PE antibody. Cells were analysed by flow cytometry. Bars represent mean ±SD of four independent repeats. **P<0.05

RPMI8226 HeCA452 positive cells display rolling on E-Selectin under dynamic flow conditions which can be inhibited by GMI1271

RPMI8226 cells were sorted into Heca452-PE positive and negative fractions. Sorted cells were treated for 1 h with 10 µM of GMI1270 and rolling assay was performed on E-Selectin coated microchannels at shear stress of 0.5 dyn/cm². No rolling was observed in the Heca452-PE negative fraction. Lines represent mean ±SD of one repeat done in triplicate. Symbols represent individual repeats.

GMI-1271 significantly enhances the activity of bortezomib in vivo

SCID-beige mice were injected with luciferase-expressing MM1S cells. Seventy two hours after inoculation, mice were divided into cohorts treated with vehicle, GMI-1271 (40mg/kg bd), bortezomib (0.75 mg/kg IP weekly for 4 weeks), or a combination of GMI-1271 and bortezomib. Tumor burden was determined by bioluminescence imaging and animals were followed for survival. *P<0.005

High ST3GAL6/low FUCA1 identifies a subgroup of high risk ISS/FISH patients with very poor outcome

We evaluated gene expression levels of 2 genes involved in selectin ligand expression, ST3GAL6 and FUCA1, which we have previously shown to be important in MM prognosis, in the context of ISS and FISH in the MRCIX dataset. Specifically, we analyzed the interaction of high ST3GAL6 and low FUCA1 with ISS/FISH risk, where high risk if ISS=3 along with 17pdel or t(4;14).The high ST3GAL6/low FUCA1 signature was overrepresented in high risk compared to low risk (39% vs. 13.9% p=0.01). Patients and conferred poor prognosis with a median survival of 10 months versus 33 months in the remaining 81% of high-risk patients. On multivariate analysis this pattern was independent of ISS/FISH for OS (p=0.013).

Conclusion

- A small fraction of MM cell lines express functional E-Selectin ligands, which may be higher in the hypoxic BM niche
- GMI1271 blocks rolling of HeCa452 MM cells on E-Selectin and enhances the efficacy of Bortezomib in vivo
- These data highlight the importance of E-Selectin ligands in engraftment and homing to the BM and provide a rationale for targeting E-Selectin in MM
- High ST3GAL6 + low FUCA1 identifies a subgroup of patients with high risk ISS/FISH with particularly poor outcome who may benefit from E-selectin inhibition

Acknowledgments and Disclosures

We wish to thank all the members of the different laboratories Magnani is an employee of GlycoMimetics Inc O’Dwyer and Gobrial: GlycoMimetics: Consultancy, Research Funding.

Acknowledgments and Disclosures