Hijacking myeloma metabolism to target cytotoxic chemotherapy to malignant plasma cells with decreased bone marrow toxicity

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ABSTRACT

Background: Overexpression of the L-type amino acid transporter 1 (LAT1; gene name *SLC7A5*) is a common feature of many malignancies to support increased protein synthesis demand. LAT1 is expressed at low levels on normal tissues and normal plasma cells but at higher levels on myeloma plasma cells. Increased LAT1 expression is also associated with decreased survival in myeloma patients. We have taken advantage of this metabolic dependency in myeloma to develop the novel therapeutic agent QBS10072S (QBS'72S) and a series of second-generation analogs. These molecules allow for selective LAT1-dependent transport of a nitrogen mustard agent into myeloma plasma cells for tumor eradication, while sparing normal hematopoietic cells when compared to nitrogen mustards used in myeloma therapy.

Results: In initial experiments, at identical doses to the nitrogen mustard melphalan, QBS'72S demonstrated reduced toxicity versus normal hematopoietic stem cells *ex vivo*. Decreased myelosuppression was found in Sprague-Dawley rats treated with QBS'72S compared to identical melphalan doses. QBS'72S monotherapy induced apoptosis in a panel of HMCLs *in vitro*. In luciferase-labeled U266 cells injected I.V. into NSG mice, QBS'72S showed significantly increased survival and decreased tumor burden versus both vehicle and bortezomib at the maximal tolerated dose. We found a similar DNA damage and apoptotic response to both QBS'72S and melphalan as evidenced by RNA-seq transcriptional signature, phosphorylation of DNA-damage associated proteins, and generation of cleaved caspase 3. Further structure-activity optimization identified next-generation analogs with even more potent anti-MM effects, greater LAT1 selectivity, and less toxicity versus normal cells.

Conclusion: Targeting myeloma metabolism via LAT1 is a promising novel strategy in myeloma, allowing for delivery of efficacious doses of nitrogen mustard to tumor with reduced myelosuppression.

DISCLOSURES

B.J., W-N.F., and K.J.K. are all employees and shareholders of Quadriga BioSciences, Inc. A.P.W. has received research funding from Quadriga BioSciences, Inc. The other authors declare no conflicts of interest.

MATERIALS AND METHODS

Colony-forming assays were performed with CD34+ stem cells isolated from normal donor bone marrow. Human myeloma cell lines (HMCLs) were treated with QBS'72S under standard conditions in 384-well plates and viability measured by CellTiterGlo assay. *In vivo* tumor burden was measured by bioluminescence imaging. RNA-seq data was analyzed using HiSat2 alignment and Cufflinks analysis. LLC-PK1 cells were single-cell cloned for stable expression of doxycycline-inducible LAT1 or LAT2 after transfection.

RESULTS

LAT1 expression is related to MM survival and progression

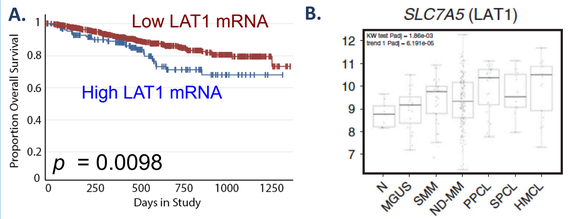


Figure 1. A. Expression of LAT1 mRNA, a known downstream target of c-myc, is correlated with overall survival in MM patients. Data from CoMMpass study[1]; cutoff for high and low expression of *SLC7A5* gene FPKM = 76. **B.** LAT1 expression is increased in malignant versus normal plasma cells (PCs) (N = normal PCs; SMM = smoldering MM; ND-MM = newly diagnosed MM; PPCL = primary plasma cell leukemia; SPCL = secondary plasma cell leukemia; HMCL = human myeloma cell lines.) Figure adapted from ref 2.

QBS'72S has anti-myeloma activity *in vitro*

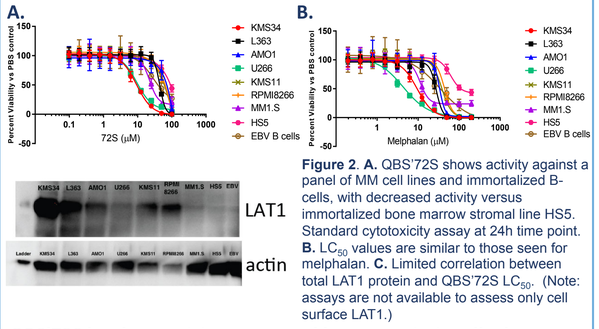


Figure 2. A. QBS'72S shows activity against a panel of MM cell lines and immortalized B-cells, with decreased activity versus immortalized bone marrow stromal line HS5. Standard cytotoxicity assay at 24h time point. **B.** LC_{50} values are similar to those seen for melphalan. **C.** Limited correlation between total LAT1 protein and QBS'72S LC_{50}. (Note: assays are not available to assess only cell surface LAT1.)

QBS'72S has less toxicity vs. normal hematopoietic cells than melphalan

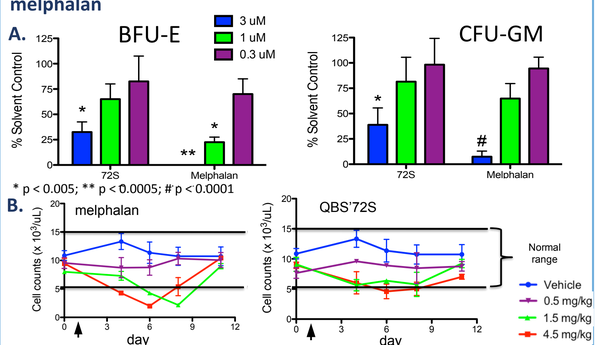


Figure 5. A. *Ex vivo* assays versus hematopoietic precursors (BFU-E = erythroid blast-forming units; CFU-GM = granulocytic/monocytic colony forming units) demonstrate reduced toxicity with QBS'72S vs. melphalan. **B.** Sprague-Dawley rats show reduced WBC depression after single dose of QBS'72S versus melphalan.

QBS'72S has potent anti-myeloma effects *in vivo*

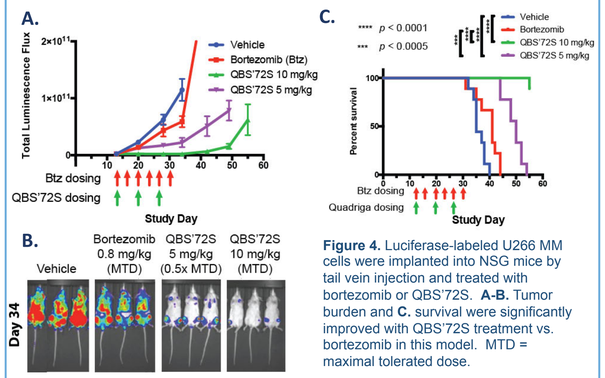


Figure 4. Luciferase-labeled U266 MM cells were implanted into NSG mice by tail vein injection and treated with bortezomib or QBS'72S. **A-B.** Tumor burden and **C.** survival were significantly improved with QBS'72S treatment vs. bortezomib in this model. MTD = maximal tolerated dose.

QBS'72S induces the DNA damage response

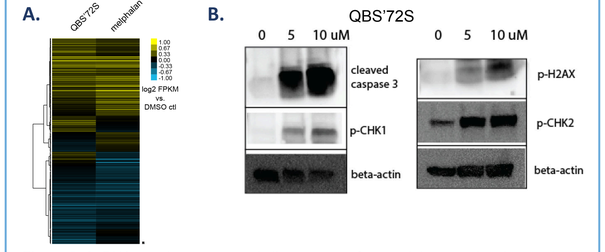
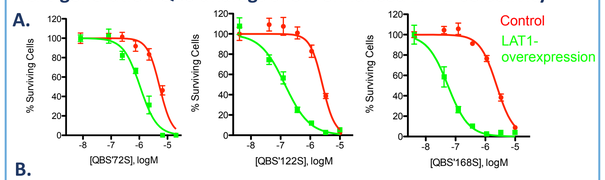


Figure 5. A. RNA-seq demonstrates similar patterns of up- and downregulated genes in U266 cells treated with 5 uM QBS'72S and melphalan vs. DMSO controls, with no statistically significant differences of any transcripts. **B.** Western blotting indicates activation of DNA damage response in U266 cells treated with QBS'72S at 24h.

Next-generation QBS analogs show enhanced LAT1-selectivity



B.

Compound	LAT1 IC_{50} (uM)	LAT2 IC_{50} (uM)	Fold LAT1-selectivity
QBS'72S	21	1100	50
QBS'122S	5.6	1500	270
QBS'168S	1.9	1500	790
Melphalan	170	390	2.3

Figure 6. A. Normal renal tubule (LLC-PK1) cells were engineered to overexpress LAT1. QBS compounds were assessed for viability effect vs. control (red) or LAT1-expressing cells (green). **B.** Compounds were tested for their ability to inhibit the uptake of ^{3}H-substrate (gabapentin or leucine, respectively) into high LAT1 or LAT2 (*SLC7A8* gene) expressing LLC-PK1 cells. QBS analogs show much greater LAT1 selectivity than melphalan.

Next-generation QBS analogs have improved toxicity profile vs. normal cell types

Compound	*In vitro* Cytotoxicity (LC_{50}, uM)			
	Normal Human Astrocytes	Mouse Fibroblasts (3T3)	Bone Marrow Progenitors Myeloid	Bone Marrow Progenitors Erythroid
QBS'72S	19	9	2.2	2.1
QBS'122S	17	12	3.1	1.5
QBS'168S	24	14	12	5.8
Melphalan	0.9	2	1.4	0.42

Figure 7. LC_{50} values for QBS analogs and melphalan versus normal cell types

Modified viability assay demonstrates next-generation QBS analogs have more potent anti-MM activity

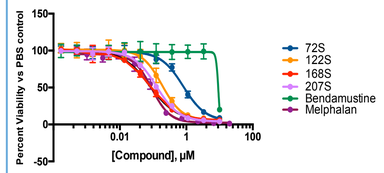


Figure 8. A modified viability assay in U266 cells, with 30 min pre-incubation of drug in PBS, without competing LAT1 amino acid substrates in media, demonstrates significantly reduced LC_{50} for QBS compounds vs. standard assay (as in Fig. 2).

SUMMARY

- Exploiting overexpression of LAT1, an amino acid transporter that enables malignant plasma cell growth, is a promising therapeutic strategy in multiple myeloma
- Transport of DNA-damaging agents into malignant plasma cells via a LAT1-selective moiety leads to potent anti-myeloma effects while reducing toxicity versus normal hematopoietic cells.
- QBS analogs with even greater selectivity for LAT1 transport hold particular promise for further preclinical development.

FUTURE DIRECTIONS

- *In vivo* comparison of second-generation QBS compound efficacy and bone marrow toxicity versus melphalan in disseminated murine MM models.
- Analysis of anti-MM effects with genetic modulation of LAT1.

REFERENCES

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2. Bolzoni et al. Dependence on glutamine uptake and glutamine addiction characterize myeloma cells: a new attractive target. *Blood* (2016) 128:667

ACKNOWLEDGEMENTS

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