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Inhibiting fatty acid binding proteins decreases multiple myeloma cell proliferation and increases efficacy of dexamethasone

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ABSTRACT

Multiple myeloma (MM) is an incurable cancer of the plasma cell and currently only has a 5 year survival rate of 53%. MM cells depend on a multitude of cells within the bone marrow microenvironment to flourish and resist treatment-induced cell death. Bone marrow adipocytes (BMAd), which increase in number with aging and obesity, have been shown to support myeloma cells by inducing proliferation, migration, and drug resistance, and ultimately contributing to myeloma patient relapse from remission. Herein we confirm the pro-myeloma effects of BMAd conditioned media (CM) and investigate the effects of the family of proteins termed the fatty acid binding proteins (FABPs), which are expressed both by adipocytes and tumor cells themselves. We found that high levels of FABP5 in patient myeloma cells corresponds to poor overall and relapse free survival for MM patients. Moreover, we found that pharmacologically inhibiting fatty acid binding proteins negatively impact tumor burden *in vitro* and *in vivo*, ultimately leading to increased survival of tumor-bearing mice. In addition, when combined with FABP inhibitors, dexamethasone, a common anti-myeloma treatment, has increased efficacy *in vitro*. Overall, these data suggest that FABPs are a novel target in myeloma and that this is a potential new cancer therapeutic target should be developed further.

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INTRODUCTION

Multiple myeloma (MM) has been characterized as a clonal expansion of malignant plasma cells, and accounts for approximately 10% of hematological neoplasms (Rajkumar, 2020). In the United States, myeloma is the ninth most common cause of cancer related deaths among females and fourteenth among males (Alexander et al., 2007). Myeloma cells home to the bone marrow (BM), leading to aberrant growth and destruction of the BM microenvironment, often resulting in painful osteolytic lesions (Fairfield, Falank, Avery, & Reagan, 2016; Falank, Fairfield, Farrell, & Reagan, 2017; Reagan & Rosen, 2015). It has been extensively demonstrated that the BM niche supports myeloma migration, invasion, proliferation and drug resistance (Fairfield et al., 2016; Reagan, Liaw, Rosen, & Ghobrial, 2015). Treatments for myeloma patients have greatly improved within the past two decades improving the five year survival rate to 53.9% ("Myeloma — Cancer Stat Facts," n.d.). In fact, modern therapies have led to >60% of newly-diagnosed multiple myeloma (NDMM) patients achieving complete response (CR) (Landgren & Iskander, 2017). While there has been an increase in therapies available to myeloma patients, most patients relapse and succumb to the disease, demonstrating the need to pursue more novel treatments for MM.

Obesity and aging are two major risk factors for development and progression of myeloma. Incidence rates of developing MM increase after the age of 40, with 2.1 persons per 100,000 person-years for individuals under the age of 65 and 30.1 persons per 100,000 person-years after the age of 65 (Alexander et al., 2007). Several studies found positive associates between obesity or high BMI and MM (Marinac et al., 2019). Interestingly, Bullwinkle and colleagues found that an increase in BMI of 5 kg/m

increases the risk of MM by 10%. It has also been shown that high BMI correlates with poor response to treatment (Bullwinkle et al., 2016; Z. Liu et al., 2015). Both aging and obesity have been shown to cause elevated amounts of adipose tissue within the BM (Bornstein et al., 2017; Hardaway, Herroon, Rajagurubandara, & Podgorski, 2014; Veldhuis-Vlug & Rosen, 2018). In fact, normal healthy BM in adults consists of more than 50% bone marrow adipose tissue (BMAT) and more than 70% of the cavity in elderly patients (Veldhuis-Vlug & Rosen, 2018). BM adipocytes (BMAd) have been shown to be supportive of myeloma cell proliferation, aggression and drug resistance in many recent publications, and suggests that targeting of BMAd would increase the longevity of MM patients (Bullwinkle et al., 2016; Fairfield et al., 2020; Z. Liu et al., 2015; Morris et al., 2020; Trotter et al., 2016).

The fatty acid bind protein (FABP) family is composed of small molecular weight, 14-15 kDa, proteins that can bind to hydrophobic ligands to contribute to transportation and storage of lipids or influence signaling pathways. There are 10 isoforms within the FABP family, and these are expressed in specific tissues. Atypical expression of FABPs has also been linked to cancer. FABP4 overexpression drives proliferation of acute myeloid leukemia, prostate and breast cancer (Guaita-Esteruelas et al., 2016; Herroon et al., 2013; Shafat et al., 2017). FABP5, epidermal FABP/mal1, expression influences prostate metastasis, clear cell renal cell carcinoma and indicates poor prognosis in breast cancer (Carbonetti et al., 2019; R. Z. Liu et al., 2011; Lv et al., 2019). FABP7, or brain FABP, has been linked to numerous neoplasms such as breast cancer, melanoma, glioblastoma, and colon cancer (Ma et al., 2018). There are many inhibitors that have been used in the literature to target FABPs, however, two that are used the most are BMS309403 (BMS)

and SBFI-26 (SBFI). BMS is typically used as an inhibitor against FABP4, while SBFI has been studied more in the context of FABP5. Both of these inhibitors bind to the binding pocket of FABPs and inhibit signaling and transportation of lipid cargo (Al-Jameel et al., 2017, 2019; Huang et al., 2017; Laouirem et al., 2019; Lee et al., 2011; Mukherjee et al., 2020). Many studies demonstrate that increased FABP expression in tumor cells leads to poorer clinical outcomes.

FABPs are influential in a multitude of different facets in malignant cells, but have yet to be studied in MM. In a recent publication, researchers demonstrated that FABP4 was released from BMAd, taken up by AML cells, and resulted in an increase in tumor cell proliferation and drug resistance (Shafat et al., 2017). The pro-tumor role of FABPs is also supported by a publication on prostate cancer and FABP4 signaling (Herroon et al., 2013). We recently published that BMAd support myeloma cell drug resistance in transwell co-culture, suggesting that factors coming from BMAd supported MM cell dexamethasone drug response (Fairfield et al., 2020). We hypothesized that BMAd secreted factors, especially FABPs, support myeloma cell dexamethasone resistance, and that inhibiting FABPs would have a negative effect on MM growth. Herein we are the first to have investigated the role of FABPs in MM cell signaling with commonly used pharmacological inhibitors *in vitro* and *in vivo*.

MATERIALS AND METHODS

Cell Culture

Human myeloma cell lines GFP^+/Luc^+MM1S (MM1S), Luc^+RPMI-8226 (ATCC, Manassas, VA) and $mCherry^+/Luc^+$OPM2 (OPM2) were maintained in RPMI 1640 medium supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA) and 1X

Antibiotic-Antimycotic (100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone) (ThermoFisher Scientific, Grand Island, NY). MM1S and OPM2 cells were generously provided by Dr. Irene Ghobrial (Dana-Farber Cancer Institute, Boston, MA). Human mesenchymal stromal cells (hMSCs) were isolated from the BM from de-identified normal patients through the Maine Medical Center (MMC) Biobank and differentiated into mature adipocytes for 21 days with an adipogenic cocktail as previously described (Fairfield et al., 2019). BM adipocyte-derived conditioned media (BMAd CM) was generated by collecting media after 48 hours from BMAds grown in basal myeloma media described above (RPMI 1640, 10% FBS, 1X Antibiotic-Antimycotic). BMAd CM was then used experimentally at a 50:50 ratio of basal media:BMAd CM by applying this to tumor cells for 72 hours.

Materials and Reagents

Recombinant FABP4 and FABP5 were purchased from Caymen Chemical (Ann Arbor, MI) and dissolved into phosphate-buffered saline. Dexamethasone (dex) was purchased from VWR, BMS3094013 (BMS) was obtained from Caymen Chemical and SBFI-26 was from Aobious (Gloucester, MA). These drugs were dissolved in DMSO to create stock solutions. *In vitro,* dexamethasone was used at 80 µM; BMS and SBFI were used at 50 µM either as single treatments or in combination. FABP4 protein level was determined using a FABP4 ELISA from R&D systems (Minneapolis, MN).

***In Vivo* Experiments and Bioluminescent Imaging**

Eight-week-old female Scid-beige mice were inoculated with 5 million MM1S cells. One cohort of mice (n=12) were administered 5 mg/kg BMS, 1 mg/kg SBFI, or the combination of the two drugs injected three times a week intraperitoneally starting one

day after tumor cell inoculation. A second cohort of mice (n=8) were administered 1 mg/kg SBFI, 1 mg/kg dexamethasone, or the combination injected three times a week intraperitoneally (i.p), starting one day after tumor cell inoculation. Mice were weighed prior to injections and were weighed throughout the experiment. Two weeks post-inoculation, tumor burden was assessed with bioluminescent imaging (BLI) biweekly as previously published (Natoni et al., 2020). In short, mice were injected with 150 mg/kg i.p. filter-sterilized D-luciferin substrate (VivoGlo, Promega) and imaged after 15 minutes in an IVIS® Lumina LT (Perkin Elmer, Inc.; Waltham, MA). Data were acquired and analyzed using LivingImage software 4.5.1. (PerkinElmer). BLI and mouse weight data were graphed and analyzed only for days in which all mice remained in the study to avoid artifacts due to mouse death. Mice were frequently monitored for clinical signs of treatment-related side effects. "Survival endpoints" were mouse death or euthanasia as required by IACUC (Body composition score depends on a single observation of >30% body weight loss, 3 consecutive measurements of >25% body weight loss, or impaired hind limb use). Survival differences were analyzed by Kaplan-Meier methodology.

Cell Number, Cell Cycle, and Apoptosis

MM cell number was measured by using BLI, Cell Titer Glo (Promega, Madison, WI), or RealTime Glo (Promega) and measured on a GLOMAX microplate reader (Promega). Cell cycle analysis was done by staining with DAPI (0.5 μg/ml). Ki67 expression was measured using Alexa fluor 647 human Ki67 antibody (BioLegend, San Diego, CA). Apoptosis was measured using Annexin V/APC and DAPI (BioLegend). All analyses were analyzed using a MACSQuant (Miltenyi Biotec, Bergisch Gladbach, Germany)

flow cytometer with a minimum of 10,000 events collected. The flow cytometric data was analyzed using FlowJo_V10 (BD Life Sciences, Ashland, OR). Analysis pathway was gating based on FSC vs SCC, doublet exclusion of SSC-H vs SSC-W for cell cycle, and then gating based of APC vs DAPI for apoptosis, DAPI histogram for cell cycle, and APC for Ki67.

Gene Expression Analyses

Basal gene expression levels of FABP family members were assessed in MM1S, OPM-2, RPMI-8226 myeloma cell lines utilizing RNA-Seq with RNA isolation, library preparation, sequencing, and analysis protocols as previously described (Fairfield et al., 2020).The Chng dataset with FABP4 and FABP5 mRNA transcript data was analyzed from accession number GEO:GSE6477 using excel and methods as previously described (Fairfield et al., 2020). The Zhan et al. dataset (GSE132604) (Zhan et al., 2006), Carrasco et al. (Carrasco et al., 2006) dataset (GEO:GSE4452), and Mulligan et al. (Mulligan et al., 2007) (GEO: GSE9782) datasets were analyzed using OncoMine (ThermoFisher) and plotted and analyzed using Graphpad Prism version 6.0 or higher.

Statistical Analysis

Data were analyzed by using Prism6 version 6.0 or higher (GraphPad). Unpaired Student's t tests or one-way or two-way analysis of variance, ANOVA, using Tukey's correction were performed. Data are expressed as mean ± standard error of the mean. ****$p \leq 0.0001$; ***$p<0.001$; **$p<0.01$; *$p<0.05$.

RESULTS

Bone marrow adipocytes support myeloma cell growth, influence cell cycle and trigger dexamethasone resistance in MM1S and OPM2 cells.

Previous publications suggested that BMAds release FABPs to support other cancer cells, such as acute myeloid leukemia, ovarian cancer, and prostate cancer (Herroon et al., 2013; Mukherjee et al., 2020; Shafat et al., 2017). Similarly, MM cells can benefit from BMAd to grow and aid in drug resistance (Fairfield et al., 2020; Z. Liu et al., 2015; Trotter et al., 2016). Thus, we investigated if BMAd conditioned media (BMAd CM) would elicit drug resistance in MM cells and if this was through FABP signaling. In a series of 72 hour experiments, cellular responses were analyzed with cell cycle, apoptosis, and Ki67 expression. BMAd CM elicited a significant increase in OPM2 cell number and a trend towards an increase in cell number in MM1S cells (Fig. 1A, D). Dexamethasone, a common anti-MM treatment, elicited a 50% reduction in cell number, but in the presence of BMAd CM, this was reduced to 10% in MM1S and OPM2 (Fig. 1B-C, E-F). MM1S Ki67 positive cells were reduced by 30% after dexamethasone treatment, but this was reduced to 10% after combination treatment with BMAd CM (Fig. 1G-H). In terms of cell cycle, dexamethasone triggered an increase in G0/G1 and a decrease in S, but this was reversed in the presence of BMAd CM (Fig. 1I). Overall, BMAd CM elicited drug resistance in MM cells and rescued the negative effect of dexamethasone on cell number.

We next investigated if BMAd CM contained FABP4. ELISA revealed that BMAd CM contained more FABP4 and that there was very little released from MM1S cells (Suppl.

Fig. 1A). Surprisingly, MM1S cells cultured in BMAd CM for 72 hours had significantly increased levels of FABP4 (Suppl. Fig. 1B). It has been noted in the literature that FABPs can compensate for one another, so we interrogated if internal FABP4 and FABP5 mRNA levels are influenced after exposure to BMAd CM. While FABP4 levels significantly increased internally in MM1S cells exposed to BMAd CM, supporting the protein data, FABP5 mRNA levels were significantly decreased by BMAd CM (Suppl. Fig. 1C-D). Interestingly, when FABP4 or FABP5 protein was added exogenously into the cultures with and without serum, MM1S and OPM2 cells did not increase their cell number (Suppl. Fig. 2A-D). Overall this suggests that exposure to BMAd CM may influence internal FABP signaling.

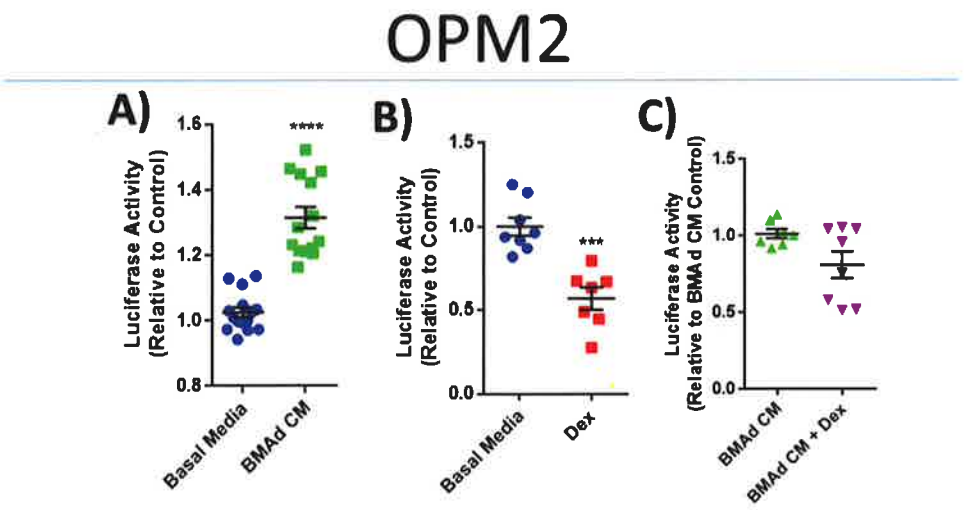
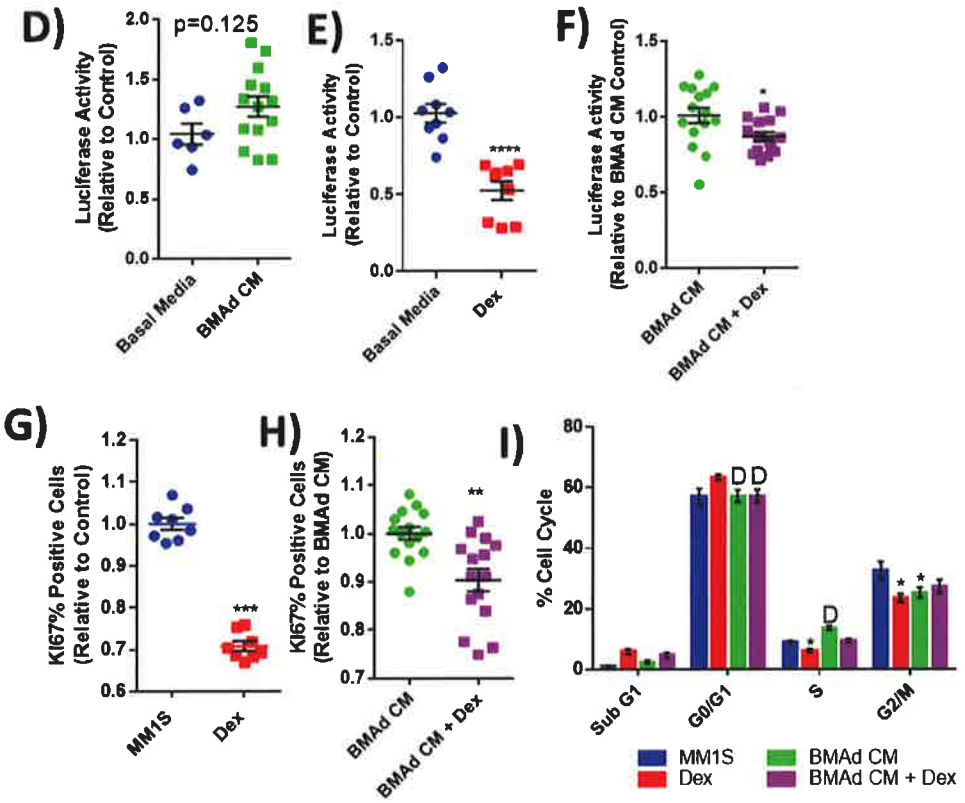


Figure 1. BMAd CM support myeloma cell and aid in evasion of dexamethasone-induced apoptosis. A) OPM2 cells increase their cell number in the presence of BMAd CM. B,C) OPM2 cell number is reduced with 80 μM dex treatment, but this is reversed in the presence of BMAd CM.

MM1S



Continued Figure 1. BMAd CM support myeloma cell and aid in evasion of dexamethasone- induced apoptosis. D) MM1S cells show a trend towards an increase in cell number in the presence of BMAd CM. E,F) MM1S cell number is reduced with 80 μM dex treatment, but this is dampened in the presence of BMAd CM. G,H) MM1S Ki67% positivity is reduced with dex treatment, but this is partially recovered with BMAd CM. I) Cell cycle was negatively impacted with dex, and partially recovered with BMAd CM.

FABP5 is highly expressed in multiple myeloma cell lines and corresponds to worse clinical outcomes in patients.

To investigate which FABPs are highly expressed in MM, we analyzed our previously published RNAseq data of three MM cells lines, OPM2, MM1S, and RPMI8226. We found that FABP5 was the most highly expressed FABP (seen in red) among all three cell lines with average expression levels of 114, 98 and 24 RPKM (Reads Per Kilobase of transcript, per Million mapped reads) in the OPM2, MM1S, and RPMI8226 cells, respectively. The second most highly expressed FABP (seen in green) was FABP6 (5.3 RPKM) in OPM2 and FABP4 in MM1S (1.8 RPKM) and RPMI8226 (1.9 RPKM) (Suppl. Table. 1) (Fairfield et al., 2020). Due to its high expression level, we analyzed the association of FABP5 with myeloma disease using independent microarray datasets from OncoMine; several datasets demonstrated a link between FABP5 and poor outcomes. Analysis of the Zhan et al. dataset indicated that patients with the highest levels of *FABP5* in MM cells had significantly shorter overall survival than patients with lowest *FABP5* expression (Zhan et al., 2006). This was true when comparing all patients, stratified as the top and bottom 100 (HR=1.322, p=0.0105) (Fig. 2A) or separated as high (n=207) or low (n=207) *FABP5* expression (HR=1.374, p=0.0105) (Fig. 2B). This result was confirmed in the Mulligan dataset (Mulligan et al., 2007) (HR=1.37, p=0.0058) and Carrasco dataset (HR =1.917, p=0.0491) (Fig. 2C-D) (Carrasco et al., 2006). Interestingly, when MM patients were classified into seven molecular subtypes based on the known genetic lesions (CD1 or CD2 of cyclin D translocation; HY: hyperdiploid; LB: low bone disease; MF or MS with activation of MAF, MAFB, or FGRF3/MMSET; PR: proliferation), patients in PR subtype, which is a signature of high-risk disease with poor

prognosis, had significantly higher expression of MM cell *FABP5* than those in the four more favorable subtypes (Zhan et al., 2006) (Fig. 2E). Moreover, in the Chng dataset, relapsed patients showed significantly increased expression of FABP5 compared to newly-diagnosed patients (Chng et al., 2007) (Fig. 2F). Immunofluorescent imaging revealed that patient samples also expressed high levels of FABP5, as demonstrated by the red staining (Fig. 2G). Additionally, immunofluorescent staining revealed that OPM2 cells express high levels of FABP5 as seen by the red staining (Fig. 2H). Overall, our data strongly suggest that FABP5 is a novel, high-risk factor in MM and targeting the FABP family may hold great promise as a new treatment avenue for MM patients.

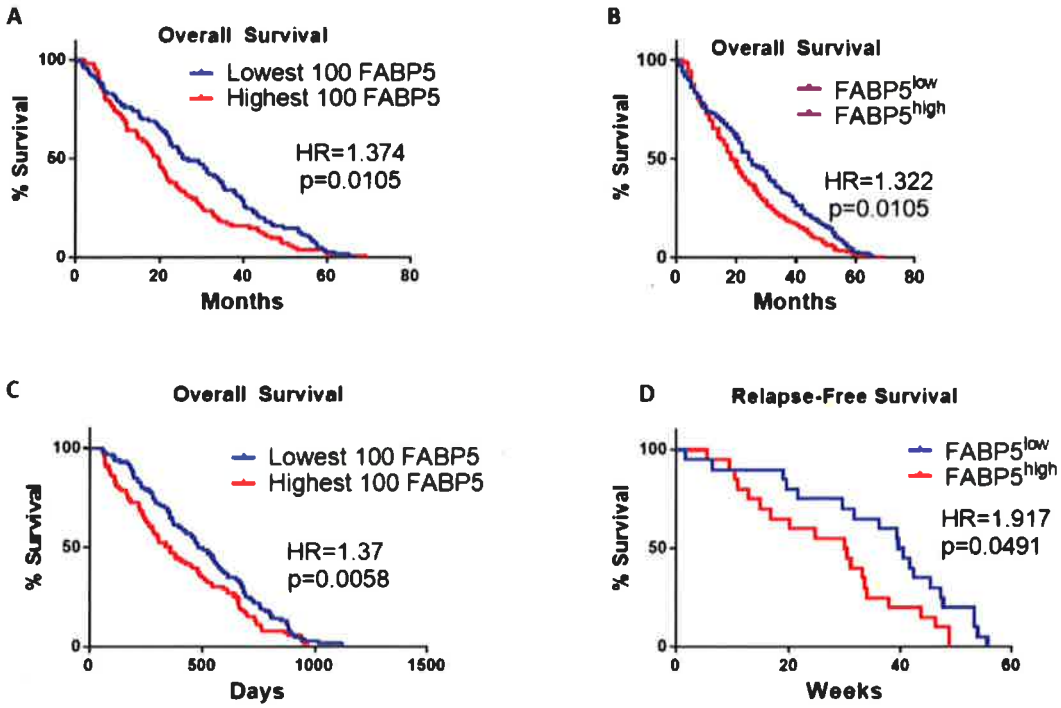
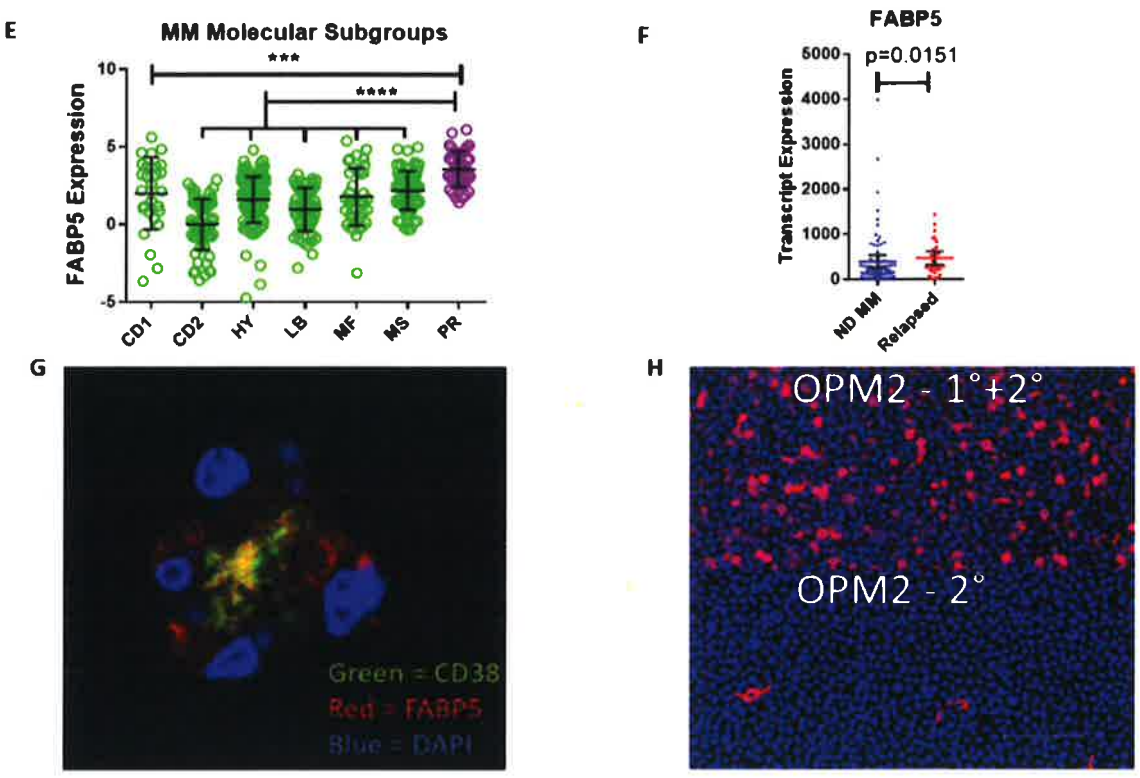


Figure 2. FABP5 expression levels corresponds to worse clinical outcomes in MM. A, B) Kaplan–Meier analysis of overall survival of myeloma patient groups in Zhan et al dataset stratified as top (n=100) or bottom (n=100) *FABP5*, or high (n=207) and low (n=207) *FABP5*. C) Kaplan–Meier analysis of overall survival of high (n=100) and low (n=100) *FABP5* expression in MM patient in Mulligan et al. dataset. D) Kaplan–Meier analysis of relapse-free survival of high (n = 20) and low (n = 20) *FABP5* expression in MM patient in Carrasco et al. dataset.

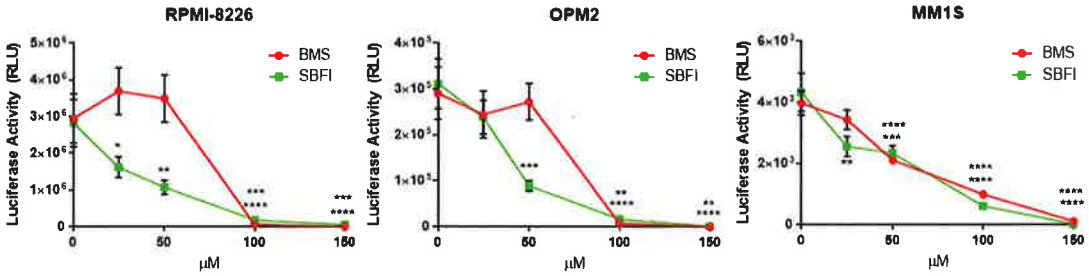


Continued Figure 2. FABP5 expression levels correspond to worse clinical outcomes in MM. E) Molecular subtypes of MM cells were analyzed for FABP5 expression and significance between all groups and the highly aggressive subtype (PR, proliferation) was observed using a One-way ANOVA with Dunnett's multiple comparison testing. F) Data is shown from Chng from newly-diagnosed (ND) (n=73) and relapsed MM patients (n=28) as mean with 95% confidence interval (CI), with statistical analysis performed using a Mann Whitney test. G) Myeloma cells from a patient stained with CD38 (green), FABP5 (red) and DAPI (blue). H) OPM2 cells stained with FABP5 (red) and DAPI (blue). Controls show the cells stained with the secondary antibody alone (2°).

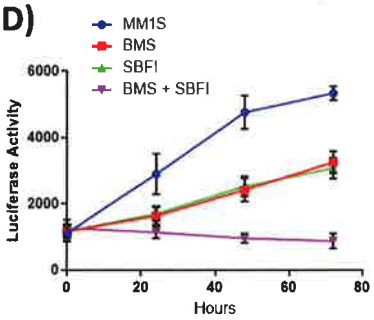
Fatty acid binding protein inhibition impairs MM cell growth and induces apoptosis in myeloma cells in a dose-dependent manner.

Next, we investigated the impact of FABP inhibition in several human MM cell lines using two well-known FABP inhibitors, BMS309403 (BMS) and SBFI-26 (SBFI). These inhibitors have been used to inhibit FABP 3, 4, 5, and 7, in several other publications, suggesting that these inhibitors could be targeting other FABPs within MM cells (Al-Jameel et al., 2017; Huang et al., 2017; Laouirem et al., 2019). However, according to RNAseq data from Fairfield et al. (Suppl. Table 1A), FABP3, 4, and 7 are expressed at a much lower level than FABP5. A 72-hour dose curve of BMS and SBFI demonstrated a decrease in RPMI8226, OPM2, and MM1S cell numbers in a dose-dependent manner. Both RPMI8226 and OPM2 had little to no luciferase activity by 100 μM, and 150 μM in the MM1S (Fig. 3A-C). To determine if this effect was specific to MM cells, human mesenchymal stem cells were exposed to similar doses, revealing no significant decrease in total ATP activity, a surrogate marker for cell number, unless at the high dose of 150 μM (data not shown). Next, we investigated how the inhibitors reduced cell numbers over time. BLI revealed that within 72 hours, the vehicle treated cells tripled in number, as expected. Single treatment of either inhibitor significantly reduced cell number compared to the control at the 72 hour time point (61%, BMS, and 57%, SBFI compared to the vehicle). Interestingly, the combination treatment significantly stunted growth (17% decrease in growth compared to day 0 seeding density) (Fig. 3D). Next, we investigated cell cycle and apoptosis using flow-based analysis to determine the influence of the FABP inhibitors. As early as 24 hours, we saw an increase in G1/G0 with the single inhibitors, with a significant increase with combination treatment. The increase in G1/G0

persisted throughout the 72 hour time course. Additionally, we saw a significant decrease in G2/M with both single inhibitors and the greatest reduction with the combination (Fig. 3E), overall suggesting a negative impact on cell cycle progression. In terms of apoptosis, we saw a significant effect of the combination as early as 24 hours and this persisted throughout the duration of the time course. By 72 hours, SBFI and the combination had significantly more apoptosis than the vehicle (Fig. 3F). These data suggest inhibition of FABPs significantly impairs cell cycle progression and induces apoptosis in human MM cells.

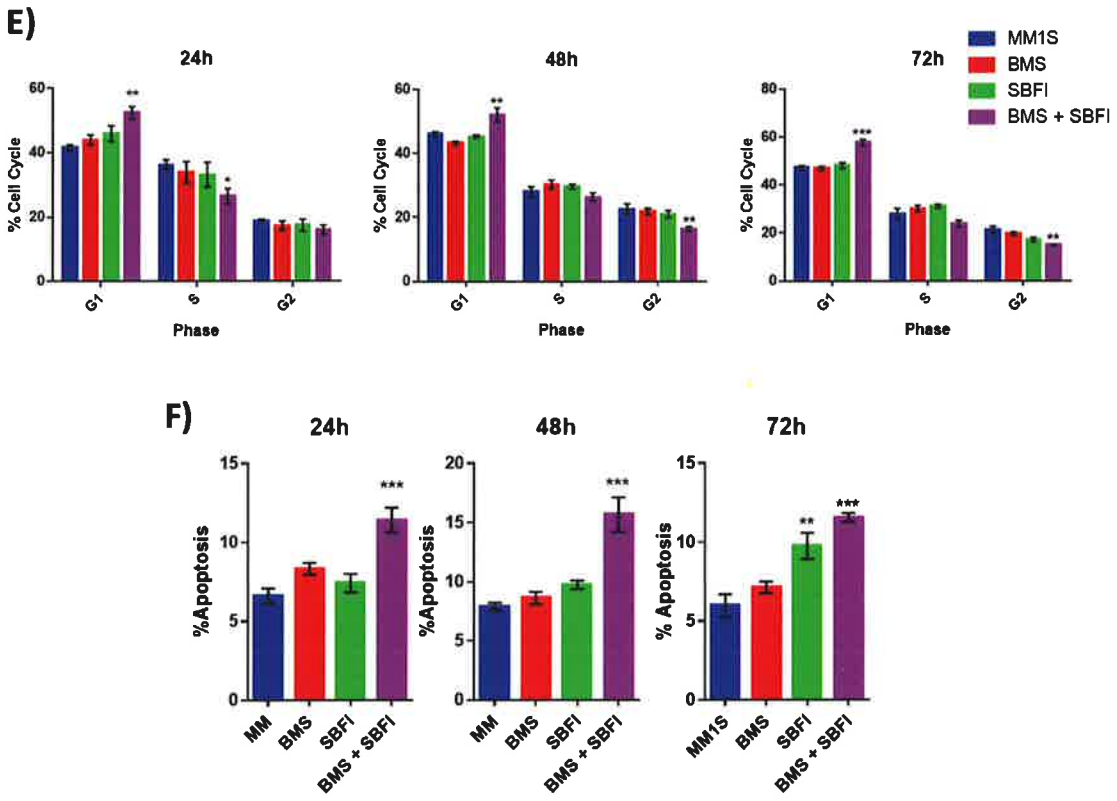


D)



Comparison	0 hours	24 hours	48 hours	72 hours
MM1S v BMS	ns	*	****	****
MM1S v SBFI	ns	*	****	****
MM1S v BMS + SBFI	ns	***	****	****
BMS v SBFI	ns	ns	ns	ns
BMS v BMS + SBFI	ns	ns	**	****
SBFI v BMS + SBFI	ns	ns	**	****

Figure 3. FABP inhibition with SBFI or BMS significantly impairs cell growth, cell cycle and induced apoptosis in MM cell lines. A-C) RPMI8226, OPM2, and MM1S cells respond to SBFI or BMS in a dose dependent manner within 72 hours. D) MM1S cells have significantly reduced cell growth over 72 hours with 50 µM BMS, 50 µM SBFI or combination treatment of both at 50 µM doses.



Continued Figure 3. FABP inhibition with SBFI or BMS significantly impairs cell growth, cell cycle and induced apoptosis in MM cell lines. E) Cell cycle and F) apoptosis is negatively impacted with 50 μM BMS, 50 μM SBFI or combination treatment of both at 50 μM in MM1S cells over a 72 hour time course.

BMS, SBFI or the combination significantly decrease tumor burden and improve survival in a multiple myeloma xenograft mouse model.

To confirm our *in vitro* findings of myeloma growth inhibition with FABP inhibitors, we moved *in vivo* to treat MM1S inoculated in 8-week-old, female Scid-beige mice. One day post-inoculation, treatment began with either BMS, SBFI or the combination three times weekly, i.p (Fig. 4A). Either single treatment or the combination did not significantly influence the weight of the mice compared to the vehicle (Fig. 4B). To monitor tumor progression, mice were subjected to bioluminescence imaging twice weekly. A significant difference in tumor burden was detected as early as day 21 in the BMS, SBFI and combination groups compared to the control. This trend in decreased tumor burden continued throughout the duration of the study for all treatment groups. At day 28, we saw significantly less tumor burden in the BMS treatment compared to either the SBFI or the combination, but this was not reflected in the survival of the mice (Fig 4C-E). In fact, mice that received BMS, SBFI or the combination had significantly longer survival than the vehicle mice, which highlights the great promise of targeting the FABPs as a potential treatment. We did not observe any negative side effects or signs of sickness from the FABP inhibitors in these mice. Overall, single or combination treatment with BMS and SBFI significantly extended the survival of the mice and reduced tumor burden in myeloma inoculated mice.

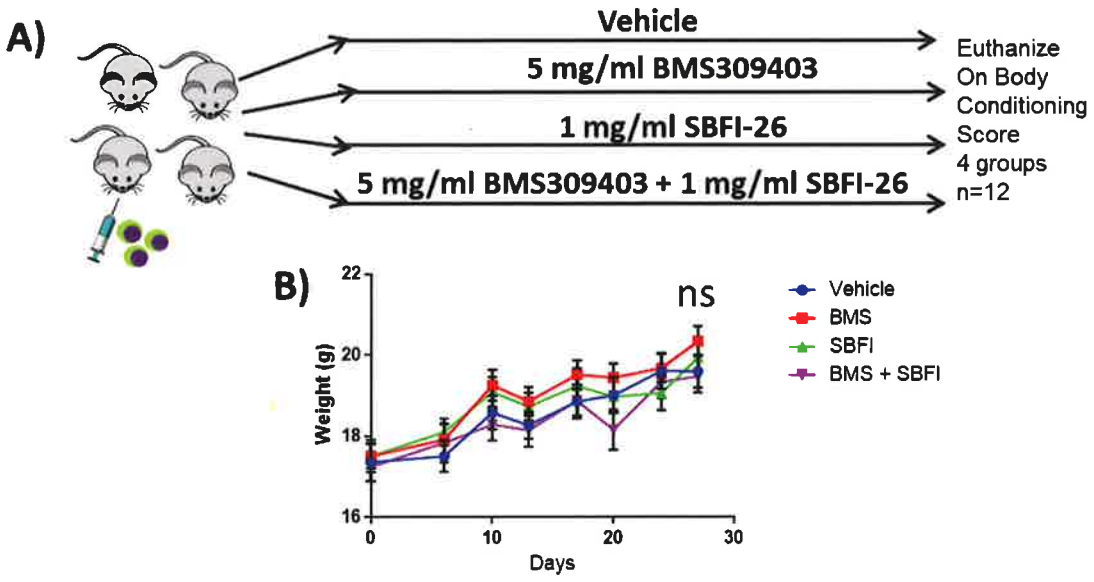
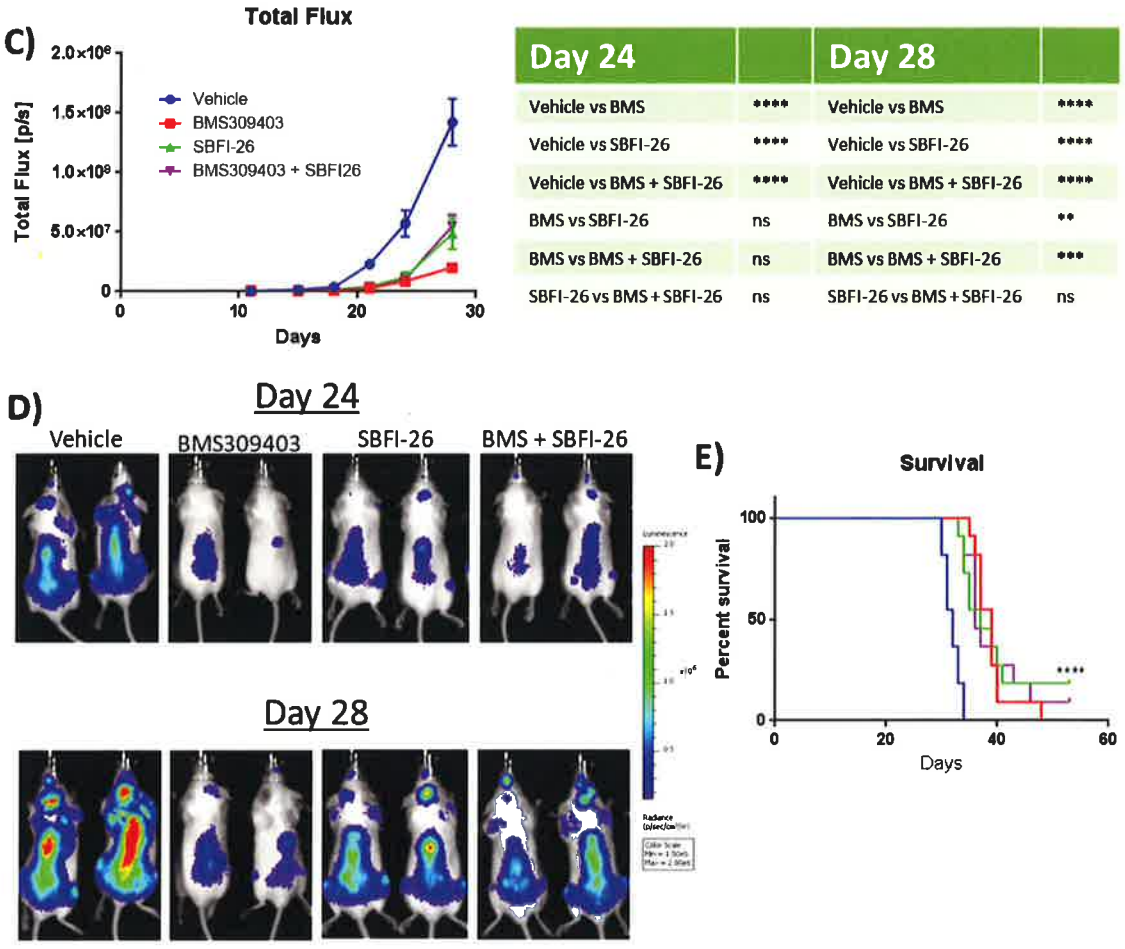


Figure 4. FABP inhibition with SBFI or BMS significantly reduces tumor burden in the MM1S xenograft model. A, B) Mice treated with the 1 mg/kg SBFI, 5 mg/kg BMS or combination treatment have no negative effect on weight. C, E) Bioluminescent imaging revealed a decrease in tumor bearing mice treated with FABP inhibitors compared to vehicle treated mice.



Continued Figure 4. FABP inhibition with SBFI or BMS significantly reduces tumor burden in the MM1S xenograft model. C-E) Bioluminescent imaging revealed a decrease in tumor bearing mice treated with FABP inhibitors compared to vehicle treated mice.

Inhibition of fatty acid binding proteins increases the efficacy of dexamethasone *in vitro* and reduces MM cell number in the presence of bone marrow adipocyte condition media.

Since we observed concurrent dexamethasone resistance and FABP4 expression in MM cells cultured in BMAd CM, and that inhibition of FABPs impacted MM cell growth, we hypothesized that inhibiting FABPs would resensitize MM cells to dexamethasone in BMAd CM conditions. In basal media, dexamethasone in combination with the single inhibitors significantly reduced cell number compared to all single treatments (~20% survival of the control). However, the greatest reduction to cell number was the combination treatment of dexamethasone, BMS and SBFI, resulting in ~5% survival (Fig. 5A). In BMAd CM conditions, there was no reduction in cell number with dexamethasone, reaffirming resistance. Single inhibitors reduced MM cell numbers compared to the BMAd CM control (BMS 33%, and SBFI 41%). In combination, BMS and SBFI significantly reduced cell number compared to the BMAd CM control (BMS + SBFI 12%) and the BMAd CM single inhibitor treatments (21% compared to BMS, and 29% compared to SBFI). Surprisingly in BMAd CM conditions, dexamethasone combined with either single inhibitors or the combination of inhibitors did not resensitize the MM cells to dexamethasone. Combination treatment of dexamethasone and BMS or SBFI had similar luciferase activity to the single inhibitors. The triple treatment had the same luciferase activity as the BMS and SBFI combination (Fig. 5B). Consistent with these findings, dexamethasone induced a 3-fold increase in apoptosis in basal conditions, but there was no increase in apoptosis with the single inhibitors (Fig. 5C). Dexamethasone co-treatment with either inhibitor resulted in significant apoptosis

compared to the control or dexamethasone alone. The combination of both inhibitors did not increase apoptosis; however, a combination of BMS, SBFI and dexamethasone triggered a 7-fold increase in apoptosis compared to the control. Importantly, dual inhibition with dexamethasone treatment significantly increased MM cell apoptosis compared to dexamethasone alone, or the single inhibitors and dexamethasone (Fig. 5C). In BMAd CM conditions, dexamethasone alone did not increase apoptosis relative to control, nor did the single inhibitors. Interestingly, triple treatment induced significantly more apoptosis compared to the control (1.8-fold), dexamethasone alone, BMS alone, or BMS and dexamethasone combination. In comparison, there was no significance between BMS alone, SBFI and dexamethasone, or BMS and SBFI (Fig. 5D, Suppl. Table 2). While FABP inhibition did not reverse drug resistance, it still reduced cell number and induced apoptosis in a rich, pro-myeloma environment.

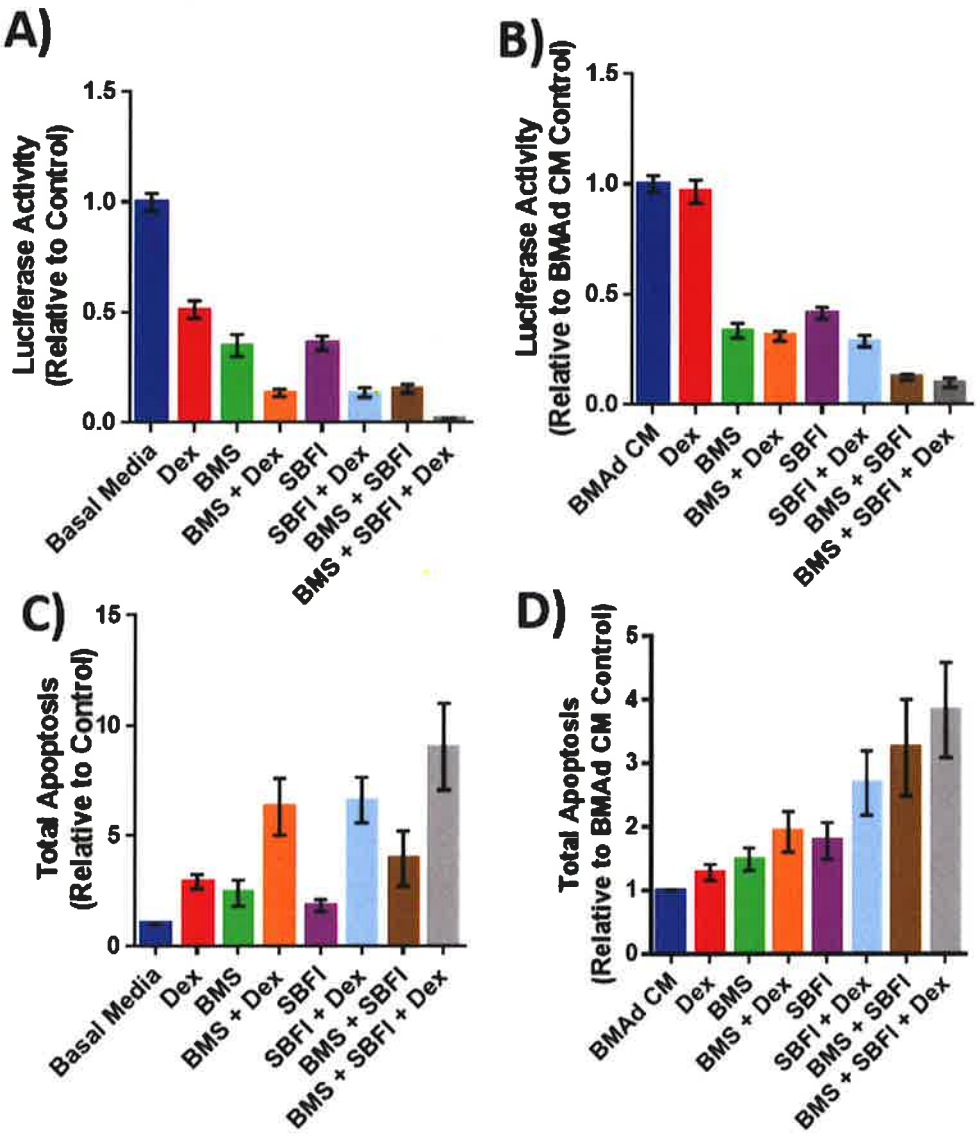
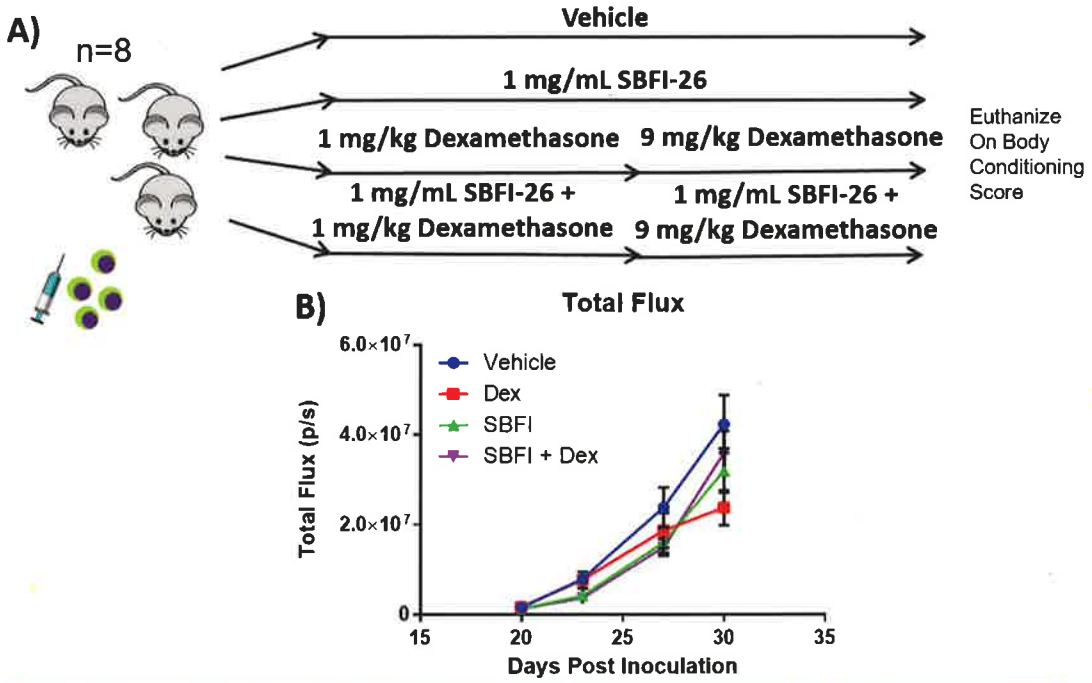


Figure 5. BMAd CM induced dexamethasone resistance in MM1S cells is not reverse with FABP inhibition. A) MM1S cells treated with 80 μM dex, 50 μM BMS, 50 μM SBFI, or the combination reduces cell number. B) BMAd CM induced dex resistance, but BMS and SBFI treatment reduces cell number. C) Dex, BMS, SBFI or the combinations induce apoptosis after 72 hour treatment in MM1S cells. D) BMAd CM induces dex resistance, but BMS, SBFI and the combinations induce apoptosis after 72 hour treatment in MM1S cells.

***In vivo* combination treatment of FABP inhibitor with dexamethasone does not reduce tumor burden.**

To test our final hypothesis that an FABP inhibitor would synergize with dexamethasone *in vivo*, we combined SBFI and dexamethasone in the Scid-beige MM1S mouse model. The treatment schedule was that either the vehicle, 1 mg/kg SBFI, 1 mg/kg dexamethasone, or the combination was administered i.p for the first three weeks of treatment. After, seeing no combined effect with this low dose of dexamethasone, the dexamethasone was increased to 9 mg/kg for the duration of the study (Fig. 6A). There were no negative side effects of the SBFI or dexamethasone treatments on weight or behavior of the mice (Suppl. Fig. 3A). BLI revealed that 1 mg/kg dexamethasone did not significantly reduce tumor burden within three weeks, but when dexamethasone was increased to 9 mg/kg, tumor burden was significantly reduced (Fig. 6B-C). Interestingly, the "SBFI only" group had a significant negative effect at day 23, which set a trend for less tumor burden throughout the study (Suppl. Fig. 3B). Surprisingly, combination treatment did not significantly reduce tumor burden compared to the vehicle, SBFI alone, or dexamethasone alone. Overall, these data suggest that while dexamethasone and SBFI may not synergize *in vivo,* SBFI is a powerful single inhibitor.



Day 20		Day 23		Day 27		Day 30	
Vehicle vs Dex	ns	Vehicle vs Dex	ns	Vehicle vs Dex	ns	Vehicle vs Dex	***
Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns
Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns
Dex vs SBFI-26	ns	Dex vs SBFI-26	ns	Dex vs SBFI-26	ns	Dex vs SBFI-26	ns
Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	*
SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns

Figure 6. SBFI is as potent as a single agent compared to combination with dexamethasone in MM1S xenograft study. A-B) MM1S xenograft mice treated with 1 mg/kg SBFI, 1 mg/kg and 9 mg/kg dex, or the combination have various responses with tumor burden.

DISCUSSION

In this thesis, I describe a series of studies where we examined the relationship between BMAd, myeloma cells and FABPs. We also studied how FABPs and BMAd can alter myeloma cell growth, apoptosis, proliferation, and response to a commonly prescribed chemotherapeutic agent, dexamethasone. We found that in MM1S and OPM2 cells, cell numbers were increased in BMAd derived conditioned media. Additionally, MM1S cells were resistant to dexamethasone in BMAd CM as shown by a rescue in their cell number, a decrease in their apoptotic response, a rescue of their cell cycle progression, and restoration of their Ki67 potential. BMAd release a high volume of FABP4, and exposure to BMAd CM elicits an increase in internal FABP4 in MM1S cells, supporting recent findings (Shu, 2020). Additionally, we observed that the fatty acid binding protein family is important to MM cell growth using 3 cell lines that represent different type of MM harboring different genetic abnormalities (RPMI8226, MM1S and OPM2 cell lines). Basally, single inhibitors of FABP4 and 5 significantly impaired cell growth over time, and this is consistent even in the presence of tumor-supportive BMAd CM. In addition, when the single inhibitors were used in combination with dexamethasone, there was a significant decrease in cell number and significantly increased apoptosis compared to dexamethasone treatment alone in basal conditions. Importantly, combination treatment of BMS, SBFI and dexamethasone resulted in the greatest reduction in cell number and significantly higher apoptosis compared to any other treatment, resulting in an almost 7-fold increase compared to the control in basal conditions. While FABP inhibition did not reverse drug resistance in BMAd CM, combining the duel inhibitors resulted in a significant increase in apoptosis and significant reduction in cell number. In summary,

these data suggest that duel inhibition of BMS and SBFI does not resensitize MM cells to dexamethasone in BMAd CM, and supports previous studies that additional adipokines are likely involved in BMAd-induced drug resistance (H. Liu et al., 2019; Z. Liu et al., 2015). A need for future studies in myeloma dexamethasone drug resistance are needed to elucidate other molecules in BMAd CM alleviating MM cells from dexamethasone-induced apoptosis (M. L. Farrell & Reagan, 2018).

Overall, targeting the FABP family appears to be a promising new target in myeloma, which could prove to be relevant in essentially all other forms of cancer. While targeting FABPs does not reverse dexamethasone resistance, targeting FABP5 looks to be clinically and translationally promising in multiple myeloma. FABP3, FABP4, FABP5, and FABP6 are expressed in three common myeloma lines, with FABP5 being the highest expressed. Clinically, high FABP5 expression correlates with poor outcomes and is significantly higher in relapsed patients. Targeting the family of FABPs with the pharmacological pan inhibitors, SBFI-26 and BMS309403, impaired myeloma growth and induced a level of apoptosis *in vitro.* Inhibition of FABPs *in vivo* significantly reduced tumor burden and extended the life span of mice. While our dexamethasone-SBFI *in vivo* experiment did not demonstrate synergy, it is possible that higher doses than we used herein could have better effects. Our data suggest that targeting FABPs could be a beneficial and important avenue to treat myeloma or other cancer patients (M. Farrell, Fairfield, D'Amico, Murphy, & Reagan, 2020).

In future studies, we plan to broaden the scope of combination treatments to extend to other anti-myeloma therapies both in terms of drug resistance and combination of FABP inhibitors. We would plan to interrogate the *in vitro* effects of BMAds and FABPs on MM resistance to proteasome inhibitors and/or immunomodulatory imide drugs, and determine the mechanisms driving this type of resistance. *In vivo,* we plan to use mouse models that have high BMAT either due to irradiation or diet, and test if MM cells are resistant to other agents beyond dexamethasone. This year, I also published as first author a manuscript showing that BM adiposity is reduced with anti-sclerostin antibody treatment, suggesting that anti-sclerostin antibody could be used in combination with dexamethasone to elucidate if MM cells respond better to dexamethasone when the microenvironment is depleted of BMAd (M. Farrell, Fairfield, Costa, et al., 2020). We are also interested in using a commonly used antibiotic, Levofloxacin, to treat myeloma burdened mice to reduce tumor growth. Levofloxacin has been showed to inhibit FABP4 activity and is currently used in the clinic for treatments in other cancers (Mukherjee et al., 2020).

Myeloma is currently incurable and the median survival is only five years, highlighting that novel new treatments are needed to expand the quality and duration of life. Our data illuminate that FABP5 is a novel therapeutic target and our findings demand more investigation into its role in myeloma due to its potential of curing, or increasing life expectancy for patients with this deadly disease.

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REFERENCES

Al-Jameel, W., Gou, X., Forootan, S. S., Al Fayi, M. S., Rudland, P. S., Forootan, F. S., … Ke, Y. (2017). Inhibitor SBFI26 suppresses the malignant progression of castration-resistant PC3-M cells by competitively binding to oncogenic FABP5. *Oncotarget*, *8*(19), 31041–31056. https://doi.org/10.18632/oncotarget.16055

Al-Jameel, W., Gou, X., Jin, X., Zhang, J., Wei, Q., Ai, J., … Ke, Y. (2019). Inactivated FABP5 suppresses malignant progression of prostate cancer cells by inhibiting the activation of nuclear fatty acid receptor PPARγ. *Genes and Cancer*, *10*(3–4), 80–96. https://doi.org/10.18632/oncoscience.192

Alexander, D. D., Mink, P. J., Adami, H. O., Cole, P., Mandel, J. S., Oken, M. M., & Trichopoulos, D. (2007, January 1). Multiple myeloma: A review of the epidemiologic literature. *International Journal of Cancer*, Vol. 120, pp. 40–61. https://doi.org/10.1002/ijc.22718

Bornstein, S., Moschetta, M., Kawano, Y., Sacco, A., Huynh, D., Brooks, D., … Reagan,

M. R. (2017). Metformin Affects Cortical Bone Mass and Marrow Adiposity in Diet-Induced Obesity in Male Mice. *Endocrinology*, *158*(10), 3369–3385. https://doi.org/10.1210/en.2017-00299

Bullwinkle, E. M., Parker, M. D., Bonan, N. F., Falkenberg, L. G., Davison, S. P., & Decicco-Skinner, K. L. (2016). *Adipocytes contribute to the growth and progression of multiple myeloma: Unraveling obesity related differences in adipocyte signaling*. https://doi.org/10.1016/j.canlet.2016.06.010

Carbonetti, G., Wilpshaar, T., Kroonen, J., Studholme, K., Converso, C., d'Oelsnitz, S., & Kaczocha, M. (2019). FABP5 coordinates lipid signaling that promotes prostate cancer metastasis. *Scientific Reports*, *9*(1), 18944. https://doi.org/10.1038/s41598-019-55418-x

Carrasco, D. R., Tonon, G., Huang, Y., Zhang, Y., Sinha, R., Feng, B., … DePinho, R. A. (2006). High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell*, *9*(4), 313–325. https://doi.org/10.1016/j.ccr.2006.03.019

Chng, W. J., Kumar, S., Vanwier, S., Ahmann, G., Price-Troska, T., Henderson, K., … Fonseca, R. (2007). Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. *Cancer Research*, *67*(7), 2982–2989. https://doi.org/10.1158/0008-5472.CAN-06-4046

Fairfield, H., Dudakovic, A., Khatib, C. M., Farrell, M., Costa, S., Falank, C., … Reagan, M. R. (2020). Myeloma-modified adipocytes exhibit metabolic dysfunction and a senescence-associated secretory phenotype (SASP). *Cancer Research*, *In Press*. https://doi.org/10.1158/0008-5472.CAN-20-1088

Fairfield, H., Falank, C., Avery, L., & Reagan, M. R. (2016). Multiple myeloma in the marrow: pathogenesis and treatments. *Annals of the New York Academy of Sciences*, *1364*(1), 32–51. https://doi.org/10.1111/nyas.13038

Fairfield, H., Falank, C., Farrell, M., Vary, C., Boucher, J. M., Driscoll, H., … Reagan, M. R. (2019). Development of a 3D bone marrow adipose tissue model. *Bone*, Vol. 118, pp. 77–88. https://doi.org/10.1016/j.bone.2018.01.023

Falank, C., Fairfield, H., Farrell, M., & Reagan, M. R. (2017). New Bone Cell Type Identified As Driver of Drug Resistance in Multiple Myeloma: The Bone Marrow Adipocyte. *Blood*, *130*(Suppl 1), 122.

Farrell, M., Fairfield, H., Costa, S., D'Amico, A., Falank, C., Brooks, D. J., & Reagan, M. R. (2020). Sclerostin-Neutralizing Antibody Treatment Rescues Negative Effects of Rosiglitazone on Mouse Bone Parameters . *Journal of Bone and Mineral Research*. https://doi.org/10.1002/jbmr.4170

Farrell, M., Fairfield, H., D'Amico, A., Murphy, C. S., & Reagan, M. R. (2020). Targeting Bone Marrow Adipose Tissue and the FABP Family Increases Efficacy of Dexamethasone in Multiple Myeloma. Retrieved December 14, 2020, from 62nd ASH Annual Meeting and Exposition website: https://annualmeeting.hematology.org/posters/25578560/Targeting-Bone-Marrow-Adipose-Tissue-and-the-FABP-Family-Increases-Efficacy-of-Dexamethasone-in-Multiple-Myeloma

Farrell, M. L., & Reagan, M. R. (2018). Soluble and cell-cell-mediated drivers of proteasome inhibitor resistance in multiple myeloma. *Frontiers in Endocrinology*, *9*(MAY). https://doi.org/10.3389/fendo.2018.00218

Guaita-Esteruelas, S., Bosquet, A., Saavedra, P., Gumà, J., Girona, J., Lam, E. W.-F., … Masana, L. (2016). Exogenous FABP4 increases breast cancer cell proliferation and activates the expression of fatty acid transport proteins. *Molecular Carcinogenesis*. https://doi.org/10.1002/mc.22485

Hardaway, A. L., Herroon, M. K., Rajagurubandara, E., & Podgorski, I. (2014). Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases. *Cancer Metastasis Reviews*, *33*(2–3), 527–543. https://doi.org/10.1007/s10555-013-9484-y

Herroon, M., Rajagurubandara, E., Hardaway, A. L., Powell, K., Turchick, A., Feldmann, D., & Podgorski, I. (2013). Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms. *Oncotarget*, *4*(11), 2108–2123. https://doi.org/10.18632/oncotarget.1482

Huang, M., Narita, S., Inoue, T., Koizumi, A., Saito, M., Tsuruta, H., … Habuchi, T. (2017). Fatty acid binding protein 4 enhances prostate cancer progression by upregulating matrix metalloproteinases and stromal cell cytokine production. *Oncotarget*, *8*(67), 111780–111794. https://doi.org/10.18632/oncotarget.22908

Landgren, O., & Iskander, K. (2017). Modern multiple myeloma therapy: deep, sustained treatment response and good clinical outcomes. *Journal of Internal Medicine*, *281*(4), 365–382. https://doi.org/10.1111/joim.12590

Laouirem, S., Sannier, A., Norkowski, E., Cauchy, F., Doblas, S., Rautou, P. E., … Paradis, V. (2019). Endothelial fatty liver binding protein 4: a new targetable mediator in hepatocellular carcinoma related to metabolic syndrome. *Oncogene*, *38*(16), 3033–3046. https://doi.org/10.1038/s41388-018-0597-1

Lee, M. Y., Li, H., Xiao, Y., Zhou, Z., Xu, A., & Vanhoutte, P. M. (2011). Chronic administration of BMS309403 improves endothelial function in apolipoprotein E-deficient mice and in cultured human endothelial cells Keywords A-FABP; endothelium-dependent relaxation; eNOS; endothelial dysfunction; nitric oxide; atherosclerosis. *1564 British Journal of Pharmacology*, *162*, 1564–1576. https://doi.org/10.1111/j.1476-5381.2010.01158.x

Liu, H., He, J., Koh, S. P., Zhong, Y., Liu, Z., Wang, Z., … Yang, J. (2019). Reprogrammed marrow adipocytes contribute to myeloma-induced bone disease. *Science Translational Medicine*, *11*(494). https://doi.org/10.1126/scitranslmed.aau9087

Liu, R. Z., Graham, K., Glubrecht, D. D., Germain, D. R., Mackey, J. R., & Godbout, R. (2011). Association of FABP5 expression with poor survival in triple-negative breast cancer: Implication for retinoic acid therapy. *American Journal of Pathology*, *178*(3), 997–1008. https://doi.org/10.1016/j.ajpath.2010.11.075

Liu, Z., Xu, J., He, J., Liu, H., Lin, P., Wan, X., … Yang, J. (2015). Mature adipocytes in bone marrow protect myeloma cells against chemotherapy through autophagy activation. *Oncotarget*, *6*(33), 34329–34341. https://doi.org/10.18632/oncotarget.6020

Lv, Q., Wang, G., Zhang, Y., Han, X., Li, H., Le, W., … Ding, Q. (2019). FABP5 regulates the proliferation of clear cell renal cell carcinoma cells via the PI3K/AKT signaling pathway. *International Journal of Oncology*, *54*(4), 1221–1232. https://doi.org/10.3892/ijo.2019.4721

Ma, R., Wang, L., Yuan, F., Wang, S., Liu, Y., Fan, T., & Wang, F. (2018). FABP7

promotes cell proliferation and survival in colon cancer through MEK/ERK signaling pathway. *Biomedicine and Pharmacotherapy*, *108*, 119–129. https://doi.org/10.1016/j.biopha.2018.08.038

Marinac, C. R., Suppan, C. A., Giovannucci, E., Song, M., Kvaerner, A. S., Townsend, M. K., … Birmann, B. M. (2019). Elucidating Under-Studied Aspects of the Link Between Obesity and Multiple Myeloma: Weight Pattern, Body Shape Trajectory, and Body Fat Distribution. *JNCI Cancer Spectrum*, *3*(3). https://doi.org/10.1093/jncics/pkz044

Morris, E. V, Suchacki, K. J., Hocking, J., Cartwright, R., Sowman, A., Gamez, B., … Edwards, C. M. (2020). Myeloma Cells Down-Regulate Adiponectin in Bone Marrow Adipocytes Via TNF-Alpha. *Journal of Bone and Mineral Research*, jbmr.3951. https://doi.org/10.1002/jbmr.3951

Mukherjee, A., Chiang, C. Y., Daifotis, H. A., Nieman, K. M., Fahrmann, J. F., Lastra, R. R., … Lengyel, E. (2020). Adipocyte-induced FABP4 expression in ovarian cancer cells promotes metastasis and mediates carboplatin resistance. *Cancer Research*, *80*(8), 1748–1761. https://doi.org/10.1158/0008-5472.CAN-19-1999

Mulligan, G., Mitsiades, C., Bryant, B., Zhan, F., Chng, W. J., Roels, S., … Anderson, K. C. (2007). Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. *Blood*, *109*(8), 3177–3188. https://doi.org/10.1182/blood-2006-09-044974

Myeloma — Cancer Stat Facts. (n.d.). Retrieved September 30, 2020, from https://seer.cancer.gov/statfacts/html/mulmy.html

Natoni, A., Farrell, M. L., Harris, S., Falank, C., Kirkham-McCarthy, L., Macauley, M. S., … O'Dwyer, M. (2020). Sialyltransferase inhibition leads to inhibition of tumor cell interactions with E-selectin, VCAM1, and MADCAM1, and improves sur vival in a human multiple myeloma mouse model. *Haematologica*, *105*(2), 457–467. https://doi.org/10.3324/haematol.2018.212266

Rajkumar, S. V. (2020). Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. *American Journal of Hematology*, *95*(5), 548–567. https://doi.org/10.1002/ajh.25791

Reagan, M. R., Liaw, L., Rosen, C. J., & Ghobrial, I. M. (2015, June 1). Dynamic interplay between bone and multiple myeloma: Emerging roles of the osteoblast. *Bone*, Vol. 75, pp. 161–169. https://doi.org/10.1016/j.bone.2015.02.021

Reagan, M. R., & Rosen, C. J. (2015). Navigating the bone marrow niche: translational insights and cancer-driven dysfunction. *Nature Reviews Rheumatology*. https://doi.org/10.1038/nrrheum.2015.160

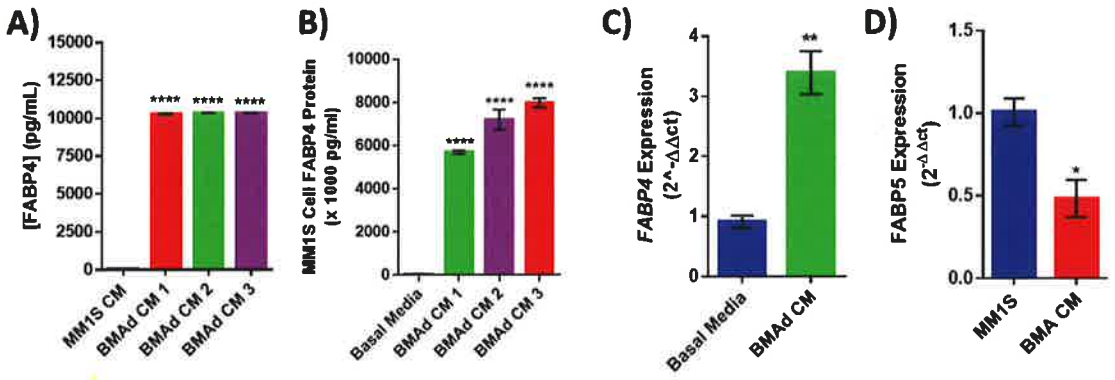
Shafat, M. S., Oellerich, T., Mohr, S., Robinson, S. D., Edwards, D. R., Marlein, C. R., … Rushworth, S. A. (2017). Leukemic blasts program bone marrow adipocytes to generate a protumoral microenvironment. *Blood*, *129*(10), 1320–1332. https://doi.org/10.1182/blood-2016-08-734798

Shu, L. (2020). Adipocyte Fatty Acid Binding Protein Promotes Multiple Myeloma through Regulating Bone Marrow Microenvironment . Retrieved December 14, 2020, from 62nd ASH Annual Meeting and Exposition website: https://annualmeeting.hematology.org/posters/25578571/Adipocyte-Fatty-Acid-Binding-Protein-Promotes-Multiple-Myeloma-through-Regulating-Bone-Marrow-Microenvironment

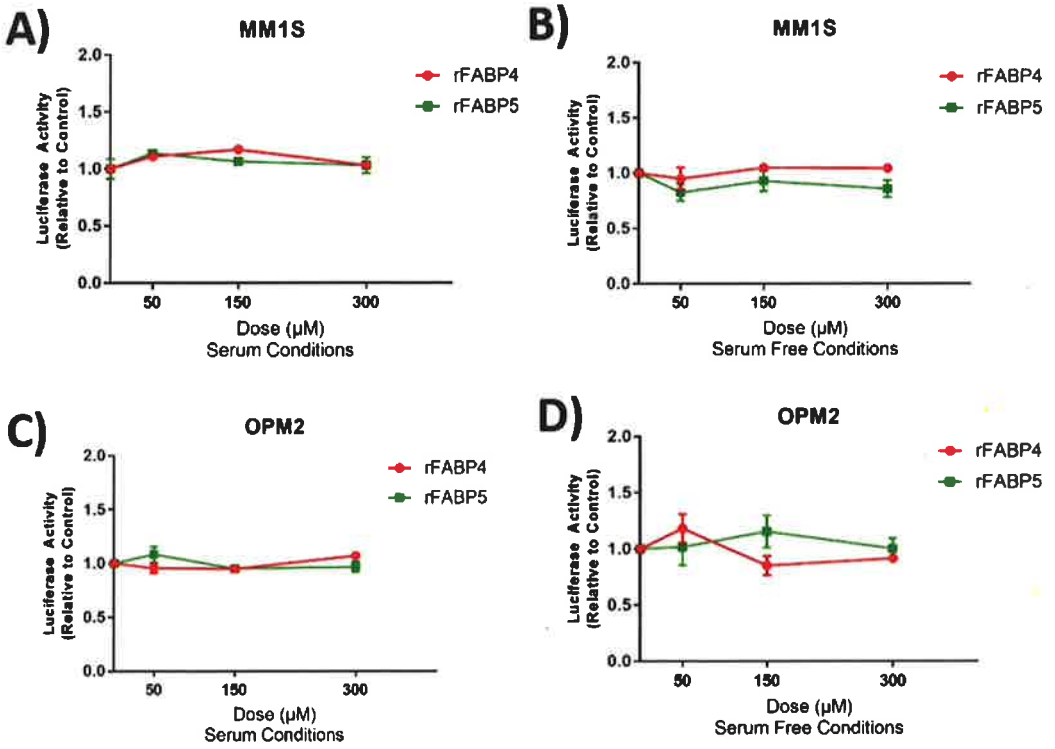
Trotter, T. N., Gibson, J. T., Sherpa, T. L., Gowda, P. S., Peker, D., & Yang, Y. (2016). Adipocyte-Lineage Cells Support Growth and Dissemination of Multiple Myeloma in Bone. *American Journal of Pathology*, *186*(11), 3054–3063. https://doi.org/10.1016/j.ajpath.2016.07.012

Veldhuis-Vlug, A. G., & Rosen, C. J. (2018, February). Clinical implications of bone marrow adiposity. *Journal of Internal Medicine*, Vol. 283, pp. 121–139. https://doi.org/10.1111/joim.12718

Zhan, F., Huang, Y., Colla, S., Stewart, J. P., Hanamura, I., Gupta, S., … Shaughnessy, J. D. (2006). The molecular classification of multiple myeloma. *Blood*, *108*(6), 2020–2028. https://doi.org/10.1182/blood-2005-11-013458



Supplemental Figure 1. FABP gene and protein expression levels are influenced by BMAd CM. A, B) FABP4 protein levels are higher in BMAd CM and in MM after BMAd CM treatment, as determined by ELISA. C) MM1S mRNA levels of FABP4 increase and D) FABP5 decrease after 72 hour treatment with



Supplemental Figure 2. Exogenously added recombinant FABP4 or 5 protein does not influence cell number. A, B) Exogenously added recombinant FABP4 or 5 protein did not influence MM1S cell number. C, D) Exogenously added recombinant FABP4 or 5 protein did not influence OPM2 cell number.

A)

GeneID (RPKM)	OPM2	MM.1S	RPMI-8226
FABP1	0	0	0
FABP2	0	0.019627	0
FABP3	1.727137	1.408361	1.710191
FABP4	1.54696	1.790967	1.946746
FABP5	114.043	98.42895	24.38133
FABP6	5.329104	1.272839	0.752652
FABP7	0	0	0
FABP9	0	0	0
FABP12	0	0	0

Supplemental Table 1. FABP5 is the highest expressed FABP in OPM2, RPMI8226 and MM1S cells. A) Basal expression levels of FABPs in OPM2, RPMI8226 and MM1S cells determined by RNAseq.

A)

Luciferase Activity - Comparison	Significance
Basal Media vs. Dex	****
Basal Media vs. BMS	****
Basal Media vs. SBFI	****
Basal Media vs. BMS + Dex	****
Basal Media vs. SBFI + Dex	****
Basal Media vs. BMS + SBFI	****
Basal Media vs. BMS + SBFI + Dex	****
Dex vs. BMS + Dex	****
Dex vs. SBFI + Dex	****
Dex vs. BMS + SBFI + Dex	****
BMS vs. BMS + Dex	***
BMS vs. BMS + SBFI	**
BMS vs. BMS + SBFI + Dex	****
SBFI vs. SBFI + Dex	***
SBFI vs. BMS + SBFI	***
SBFI vs. BMS + SBFI + Dex	****
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	ns

B)

Luciferase Activity - Comparison	Significance
BMAd CM vs. Dex	ns
BMAd CM vs. BMS	****
BMAd CM vs. SBFI	****
BMAd CM vs. BMS + Dex	****
BMAd CM vs. SBFI + Dex	****
BMAd CM vs. BMS + SBFI	****
BMAd CM vs. BMS + SBFI + Dex	****
Dex vs. BMS + Dex	****
Dex vs. SBFI + Dex	****
Dex vs. BMS + SBFI + Dex	****
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	***
BMS vs. BMS + SBFI + Dex	****
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	****
SBFI vs. BMS + SBFI + Dex	****
BMS + Dex vs. BMS + SBFI + Dex	***
SBFI + Dex vs. BMS + SBFI + Dex	**
BMS + SBFI vs. BMS + SBFI + Dex	ns

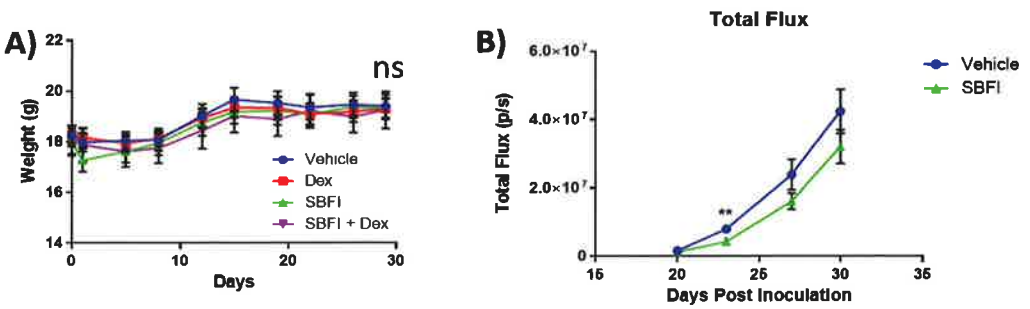
C)

Total Apoptosis- Comparison	Significance
Basal Media vs. Dex	ns
Basal Media vs. BMS	ns
Basal Media vs. SBFI	ns
Basal Media vs. BMS + Dex	*
Basal Media vs. SBFI + Dex	*
Basal Media vs. BMS + SBFI	ns
Basal Media vs. BMS + SBFI + Dex	***
Dex vs. BMS + Dex	ns
Dex vs. SBFI + Dex	ns
Dex vs. BMS + SBFI + Dex	**
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	ns
BMS vs. BMS + SBFI + Dex	**
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	ns
SBFI vs. BMS + SBFI + Dex	***
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	*

D)

Total Apoptosis- Comparison	Significance
BMAd CM vs. Dex	ns
BMAd CM vs. BMS	ns
BMAd CM vs. SBFI	ns
BMAd CM vs. BMS + Dex	ns
BMAd CM vs. SBFI + Dex	ns
BMAd CM vs. BMS + SBFI	*
BMAd CM vs. BMS + SBFI + Dex	**
Dex vs. BMS + Dex	ns
Dex vs. SBFI + Dex	ns
Dex vs. BMS + SBFI + Dex	**
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	ns
BMS vs. BMS + SBFI + Dex	*
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	ns
SBFI vs. BMS + SBFI + Dex	*
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	ns

Supplemental Table 2. Combinatorial treatment of dexamethasone, BMS and SBFI induce apoptosis and reduce cell number after 72 hour treatment in MM1S cells. A-D) Statistical importance of treatment with 50 μM BMS, 50 μM SBFI, 80 μM dex and the combinations after 72 hours in MM1S cells.



Supplemental Figure 4. SBFI, dexamethasone, or the combination treated mice maintain weight. A) No weight difference between treatment groups over the duration of study. B) SBFI treated mice have reduced tumor burden compared to vehicle treated mice.

Inhibiting fatty acid binding proteins decreases multiple myeloma cell proliferation and increases efficacy of dexamethasone

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A thesis

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DEPARTMENT OF BIOLOGICAL SCIENCES

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We hereby recommend that the thesis of entitled:

Inhibiting fatty acid binding proteins deceases multiple myeloma cell proliferation and increases efficacy of dexamethasone

Be accepted as partial fulfillment of the requirements for the degree of

Master of Science in Biology

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ABSTRACT

Multiple myeloma (MM) is an incurable cancer of the plasma cell and currently only has a 5 year survival rate of 53%. MM cells depend on a multitude of cells within the bone marrow microenvironment to flourish and resist treatment-induced cell death. Bone marrow adipocytes (BMAd), which increase in number with aging and obesity, have been shown to support myeloma cells by inducing proliferation, migration, and drug resistance, and ultimately contributing to myeloma patient relapse from remission. Herein we confirm the pro-myeloma effects of BMAd conditioned media (CM) and investigate the effects of the family of proteins termed the fatty acid binding proteins (FABPs), which are expressed both by adipocytes and tumor cells themselves. We found that high levels of FABP5 in patient myeloma cells corresponds to poor overall and relapse free survival for MM patients. Moreover, we found that pharmacologically inhibiting fatty acid binding proteins negatively impact tumor burden *in vitro* and *in vivo*, ultimately leading to increased survival of tumor-bearing mice. In addition, when combined with FABP inhibitors, dexamethasone, a common anti-myeloma treatment, has increased efficacy *in vitro*. Overall, these data suggest that FABPs are a novel target in myeloma and that this is a potential new cancer therapeutic target should be developed further.

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INTRODUCTION

Multiple myeloma (MM) has been characterized as a clonal expansion of malignant plasma cells, and accounts for approximately 10% of hematological neoplasms (Rajkumar, 2020). In the United States, myeloma is the ninth most common cause of cancer related deaths among females and fourteenth among males (Alexander et al., 2007). Myeloma cells home to the bone marrow (BM), leading to aberrant growth and destruction of the BM microenvironment, often resulting in painful osteolytic lesions (Fairfield, Falank, Avery, & Reagan, 2016; Falank, Fairfield, Farrell, & Reagan, 2017; Reagan & Rosen, 2015). It has been extensively demonstrated that the BM niche supports myeloma migration, invasion, proliferation and drug resistance (Fairfield et al., 2016; Reagan, Liaw, Rosen, & Ghobrial, 2015). Treatments for myeloma patients have greatly improved within the past two decades improving the five year survival rate to 53.9% ("Myeloma — Cancer Stat Facts," n.d.). In fact, modern therapies have led to >60% of newly-diagnosed multiple myeloma (NDMM) patients achieving complete response (CR) (Landgren & Iskander, 2017). While there has been an increase in therapies available to myeloma patients, most patients relapse and succumb to the disease, demonstrating the need to pursue more novel treatments for MM.

Obesity and aging are two major risk factors for development and progression of myeloma. Incidence rates of developing MM increase after the age of 40, with 2.1 persons per 100,000 person-years for individuals under the age of 65 and 30.1 persons per 100,000 person-years after the age of 65 (Alexander et al., 2007). Several studies found positive associates between obesity or high BMI and MM (Marinac et al., 2019). Interestingly, Bullwinkle and colleagues found that an increase in BMI of 5 kg/m

increases the risk of MM by 10%. It has also been shown that high BMI correlates with poor response to treatment (Bullwinkle et al., 2016; Z. Liu et al., 2015). Both aging and obesity have been shown to cause elevated amounts of adipose tissue within the BM (Bornstein et al., 2017; Hardaway, Herroon, Rajagurubandara, & Podgorski, 2014; Veldhuis-Vlug & Rosen, 2018). In fact, normal healthy BM in adults consists of more than 50% bone marrow adipose tissue (BMAT) and more than 70% of the cavity in elderly patients (Veldhuis-Vlug & Rosen, 2018). BM adipocytes (BMAd) have been shown to be supportive of myeloma cell proliferation, aggression and drug resistance in many recent publications, and suggests that targeting of BMAd would increase the longevity of MM patients (Bullwinkle et al., 2016; Fairfield et al., 2020; Z. Liu et al., 2015; Morris et al., 2020; Trotter et al., 2016).

The fatty acid bind protein (FABP) family is composed of small molecular weight, 14-15 kDa, proteins that can bind to hydrophobic ligands to contribute to transportation and storage of lipids or influence signaling pathways. There are 10 isoforms within the FABP family, and these are expressed in specific tissues. Atypical expression of FABPs has also been linked to cancer. FABP4 overexpression drives proliferation of acute myeloid leukemia, prostate and breast cancer (Guaita-Esteruelas et al., 2016; Herroon et al., 2013; Shafat et al., 2017). FABP5, epidermal FABP/mal1, expression influences prostate metastasis, clear cell renal cell carcinoma and indicates poor prognosis in breast cancer (Carbonetti et al., 2019; R. Z. Liu et al., 2011; Lv et al., 2019). FABP7, or brain FABP, has been linked to numerous neoplasms such as breast cancer, melanoma, glioblastoma, and colon cancer (Ma et al., 2018). There are many inhibitors that have been used in the literature to target FABPs, however, two that are used the most are BMS309403 (BMS)

and SBFI-26 (SBFI). BMS is typically used as an inhibitor against FABP4, while SBFI has been studied more in the context of FABP5. Both of these inhibitors bind to the binding pocket of FABPs and inhibit signaling and transportation of lipid cargo (Al-Jameel et al., 2017, 2019; Huang et al., 2017; Laouirem et al., 2019; Lee et al., 2011; Mukherjee et al., 2020). Many studies demonstrate that increased FABP expression in tumor cells leads to poorer clinical outcomes.

FABPs are influential in a multitude of different facets in malignant cells, but have yet to be studied in MM. In a recent publication, researchers demonstrated that FABP4 was released from BMAd, taken up by AML cells, and resulted in an increase in tumor cell proliferation and drug resistance (Shafat et al., 2017). The pro-tumor role of FABPs is also supported by a publication on prostate cancer and FABP4 signaling (Herroon et al., 2013). We recently published that BMAd support myeloma cell drug resistance in transwell co-culture, suggesting that factors coming from BMAd supported MM cell dexamethasone drug response (Fairfield et al., 2020). We hypothesized that BMAd secreted factors, especially FABPs, support myeloma cell dexamethasone resistance, and that inhibiting FABPs would have a negative effect on MM growth. Herein we are the first to have investigated the role of FABPs in MM cell signaling with commonly used pharmacological inhibitors *in vitro* and *in vivo*.

MATERIALS AND METHODS

Cell Culture

Human myeloma cell lines GFP^+/Luc^+MM1S (MM1S), Luc^+RPMI-8226 (ATCC, Manassas, VA) and $mCherry^+/Luc^+$OPM2 (OPM2) were maintained in RPMI 1640 medium supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA) and 1X

Antibiotic-Antimycotic (100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone) (ThermoFisher Scientific, Grand Island, NY). MM1S and OPM2 cells were generously provided by Dr. Irene Ghobrial (Dana-Farber Cancer Institute, Boston, MA). Human mesenchymal stromal cells (hMSCs) were isolated from the BM from de-identified normal patients through the Maine Medical Center (MMC) Biobank and differentiated into mature adipocytes for 21 days with an adipogenic cocktail as previously described (Fairfield et al., 2019). BM adipocyte-derived conditioned media (BMAd CM) was generated by collecting media after 48 hours from BMAds grown in basal myeloma media described above (RPMI 1640, 10% FBS, 1X Antibiotic-Antimycotic). BMAd CM was then used experimentally at a 50:50 ratio of basal media:BMAd CM by applying this to tumor cells for 72 hours.

Materials and Reagents

Recombinant FABP4 and FABP5 were purchased from Caymen Chemical (Ann Arbor, MI) and dissolved into phosphate-buffered saline. Dexamethasone (dex) was purchased from VWR, BMS3094013 (BMS) was obtained from Caymen Chemical and SBFI-26 was from Aobious (Gloucester, MA). These drugs were dissolved in DMSO to create stock solutions. *In vitro,* dexamethasone was used at 80 µM; BMS and SBFI were used at 50 µM either as single treatments or in combination. FABP4 protein level was determined using a FABP4 ELISA from R&D systems (Minneapolis, MN).

***In Vivo* Experiments and Bioluminescent Imaging**

Eight-week-old female Scid-beige mice were inoculated with 5 million MM1S cells. One cohort of mice (n=12) were administered 5 mg/kg BMS, 1 mg/kg SBFI, or the combination of the two drugs injected three times a week intraperitoneally starting one

day after tumor cell inoculation. A second cohort of mice (n=8) were administered 1 mg/kg SBFI, 1 mg/kg dexamethasone, or the combination injected three times a week intraperitoneally (i.p), starting one day after tumor cell inoculation. Mice were weighed prior to injections and were weighed throughout the experiment. Two weeks post-inoculation, tumor burden was assessed with bioluminescent imaging (BLI) biweekly as previously published (Natoni et al., 2020). In short, mice were injected with 150 mg/kg i.p. filter-sterilized D-luciferin substrate (VivoGlo, Promega) and imaged after 15 minutes in an IVIS® Lumina LT (Perkin Elmer, Inc.; Waltham, MA). Data were acquired and analyzed using LivingImage software 4.5.1. (PerkinElmer). BLI and mouse weight data were graphed and analyzed only for days in which all mice remained in the study to avoid artifacts due to mouse death. Mice were frequently monitored for clinical signs of treatment-related side effects. "Survival endpoints" were mouse death or euthanasia as required by IACUC (Body composition score depends on a single observation of >30% body weight loss, 3 consecutive measurements of >25% body weight loss, or impaired hind limb use). Survival differences were analyzed by Kaplan-Meier methodology.

Cell Number, Cell Cycle, and Apoptosis

MM cell number was measured by using BLI, Cell Titer Glo (Promega, Madison, WI), or RealTime Glo (Promega) and measured on a GLOMAX microplate reader (Promega). Cell cycle analysis was done by staining with DAPI (0.5 μg/ml). Ki67 expression was measured using Alexa fluor 647 human Ki67 antibody (BioLegend, San Diego, CA). Apoptosis was measured using Annexin V/APC and DAPI (BioLegend). All analyses were analyzed using a MACSQuant (Miltenyi Biotec, Bergisch Gladbach, Germany)

flow cytometer with a minimum of 10,000 events collected. The flow cytometric data was analyzed using FlowJo_V10 (BD Life Sciences, Ashland, OR). Analysis pathway was gating based on FSC vs SCC, doublet exclusion of SSC-H vs SSC-W for cell cycle, and then gating based of APC vs DAPI for apoptosis, DAPI histogram for cell cycle, and APC for Ki67.

Gene Expression Analyses

Basal gene expression levels of FABP family members were assessed in MM1S, OPM-2, RPMI-8226 myeloma cell lines utilizing RNA-Seq with RNA isolation, library preparation, sequencing, and analysis protocols as previously described (Fairfield et al., 2020).The Chng dataset with FABP4 and FABP5 mRNA transcript data was analyzed from accession number GEO:GSE6477 using excel and methods as previously described (Fairfield et al., 2020). The Zhan et al. dataset (GSE132604) (Zhan et al., 2006), Carrasco et al. (Carrasco et al., 2006) dataset (GEO:GSE4452), and Mulligan et al. (Mulligan et al., 2007) (GEO: GSE9782) datasets were analyzed using OncoMine (ThermoFisher) and plotted and analyzed using Graphpad Prism version 6.0 or higher.

Statistical Analysis

Data were analyzed by using Prism6 version 6.0 or higher (GraphPad). Unpaired Student's t tests or one-way or two-way analysis of variance, ANOVA, using Tukey's correction were performed. Data are expressed as mean ± standard error of the mean. ****$p \leq 0.0001$; ***$p<0.001$; **$p<0.01$; *$p<0.05$.

RESULTS

Bone marrow adipocytes support myeloma cell growth, influence cell cycle and trigger dexamethasone resistance in MM1S and OPM2 cells.

Previous publications suggested that BMAds release FABPs to support other cancer cells, such as acute myeloid leukemia, ovarian cancer, and prostate cancer (Herroon et al., 2013; Mukherjee et al., 2020; Shafat et al., 2017). Similarly, MM cells can benefit from BMAd to grow and aid in drug resistance (Fairfield et al., 2020; Z. Liu et al., 2015; Trotter et al., 2016). Thus, we investigated if BMAd conditioned media (BMAd CM) would elicit drug resistance in MM cells and if this was through FABP signaling. In a series of 72 hour experiments, cellular responses were analyzed with cell cycle, apoptosis, and Ki67 expression. BMAd CM elicited a significant increase in OPM2 cell number and a trend towards an increase in cell number in MM1S cells (Fig. 1A, D). Dexamethasone, a common anti-MM treatment, elicited a 50% reduction in cell number, but in the presence of BMAd CM, this was reduced to 10% in MM1S and OPM2 (Fig. 1B-C, E-F). MM1S Ki67 positive cells were reduced by 30% after dexamethasone treatment, but this was reduced to 10% after combination treatment with BMAd CM (Fig. 1G-H). In terms of cell cycle, dexamethasone triggered an increase in G0/G1 and a decrease in S, but this was reversed in the presence of BMAd CM (Fig. 1I). Overall, BMAd CM elicited drug resistance in MM cells and rescued the negative effect of dexamethasone on cell number.

We next investigated if BMAd CM contained FABP4. ELISA revealed that BMAd CM contained more FABP4 and that there was very little released from MM1S cells (Suppl.

Fig. 1A). Surprisingly, MM1S cells cultured in BMAd CM for 72 hours had significantly increased levels of FABP4 (Suppl. Fig. 1B). It has been noted in the literature that FABPs can compensate for one another, so we interrogated if internal FABP4 and FABP5 mRNA levels are influenced after exposure to BMAd CM. While FABP4 levels significantly increased internally in MM1S cells exposed to BMAd CM, supporting the protein data, FABP5 mRNA levels were significantly decreased by BMAd CM (Suppl. Fig. 1C-D). Interestingly, when FABP4 or FABP5 protein was added exogenously into the cultures with and without serum, MM1S and OPM2 cells did not increase their cell number (Suppl. Fig. 2A-D). Overall this suggests that exposure to BMAd CM may influence internal FABP signaling.

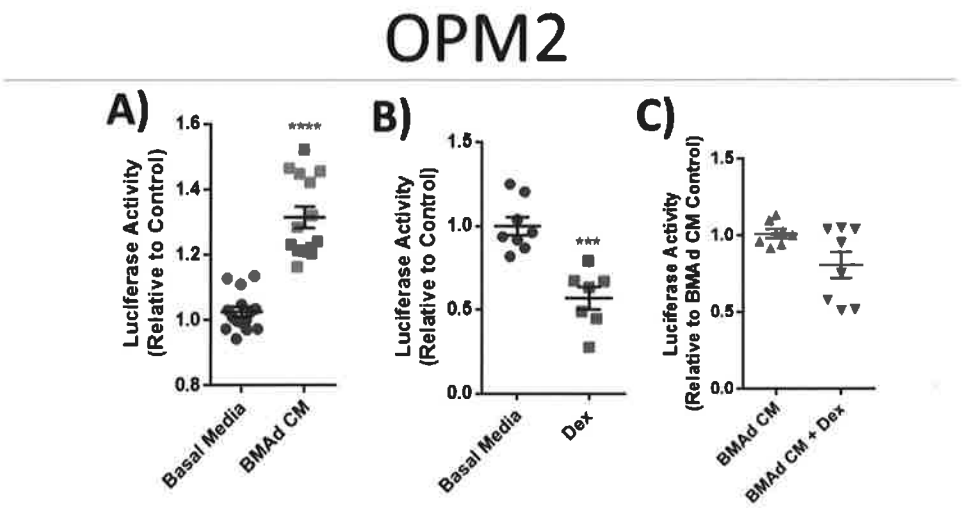
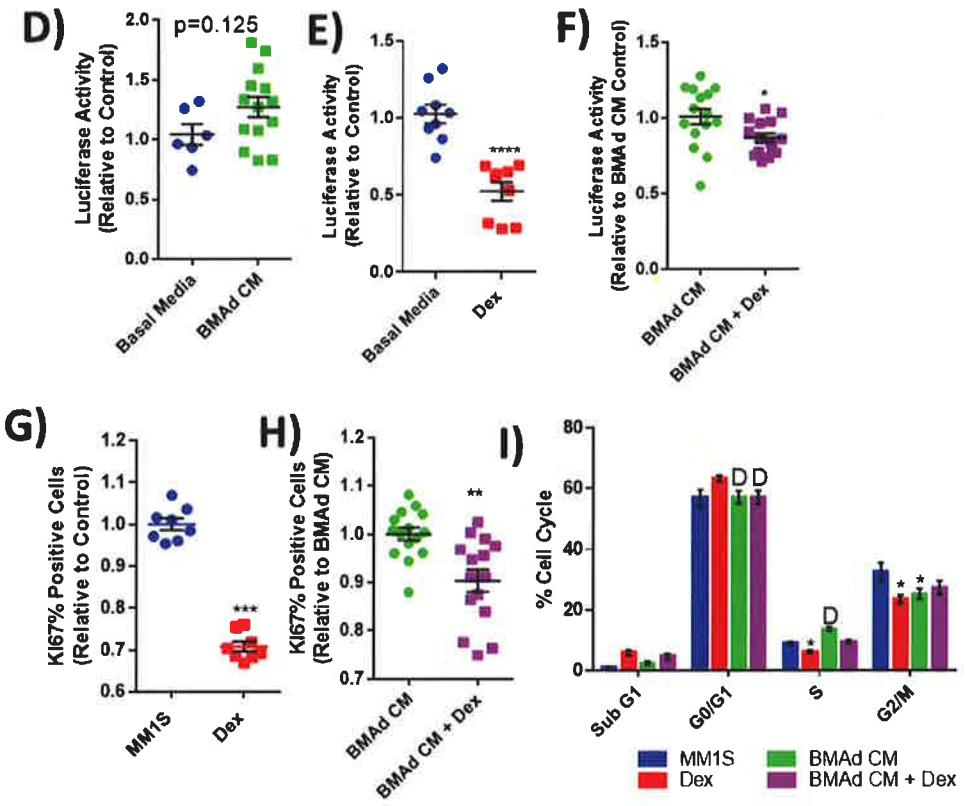


Figure 1. BMAd CM support myeloma cell and aid in evasion of dexamethasone-induced apoptosis. A) OPM2 cells increase their cell number in the presence of BMAd CM. B,C) OPM2 cell number is reduced with 80 μM dex treatment, but this is reversed in the presence of BMAd CM.

MM1S



Continued Figure 1. BMAd CM support myeloma cell and aid in evasion of dexamethasone- induced apoptosis. D) MM1S cells show a trend towards an increase in cell number in the presence of BMAd CM. E,F) MM1S cell number is reduced with 80 μM dex treatment, but this is dampened in the presence of BMAd CM. G,H) MM1S Ki67% positivity is reduced with dex treatment, but this is partially recovered with BMAd CM. I) Cell cycle was negatively impacted with dex, and partially recovered with BMAd CM.

FABP5 is highly expressed in multiple myeloma cell lines and corresponds to worse clinical outcomes in patients.

To investigate which FABPs are highly expressed in MM, we analyzed our previously published RNAseq data of three MM cells lines, OPM2, MM1S, and RPMI8226. We found that FABP5 was the most highly expressed FABP (seen in red) among all three cell lines with average expression levels of 114, 98 and 24 RPKM (Reads Per Kilobase of transcript, per Million mapped reads) in the OPM2, MM1S, and RPMI8226 cells, respectively. The second most highly expressed FABP (seen in green) was FABP6 (5.3 RPKM) in OPM2 and FABP4 in MM1S (1.8 RPKM) and RPMI8226 (1.9 RPKM) (Suppl. Table. 1) (Fairfield et al., 2020). Due to its high expression level, we analyzed the association of FABP5 with myeloma disease using independent microarray datasets from OncoMine; several datasets demonstrated a link between FABP5 and poor outcomes. Analysis of the Zhan et al. dataset indicated that patients with the highest levels of *FABP5* in MM cells had significantly shorter overall survival than patients with lowest *FABP5* expression (Zhan et al., 2006). This was true when comparing all patients, stratified as the top and bottom 100 (HR=1.322, p=0.0105) (Fig. 2A) or separated as high (n=207) or low (n=207) *FABP5* expression (HR=1.374, p=0.0105) (Fig. 2B). This result was confirmed in the Mulligan dataset (Mulligan et al., 2007) (HR=1.37, p=0.0058) and Carrasco dataset (HR =1.917, p=0.0491) (Fig. 2C-D) (Carrasco et al., 2006). Interestingly, when MM patients were classified into seven molecular subtypes based on the known genetic lesions (CD1 or CD2 of cyclin D translocation; HY: hyperdiploid; LB: low bone disease; MF or MS with activation of MAF, MAFB, or FGRF3/MMSET; PR: proliferation), patients in PR subtype, which is a signature of high-risk disease with poor

prognosis, had significantly higher expression of MM cell *FABP5* than those in the four more favorable subtypes (Zhan et al., 2006) (Fig. 2E). Moreover, in the Chng dataset, relapsed patients showed significantly increased expression of FABP5 compared to newly-diagnosed patients (Chng et al., 2007) (Fig. 2F). Immunofluorescent imaging revealed that patient samples also expressed high levels of FABP5, as demonstrated by the red staining (Fig. 2G). Additionally, immunofluorescent staining revealed that OPM2 cells express high levels of FABP5 as seen by the red staining (Fig. 2H). Overall, our data strongly suggest that FABP5 is a novel, high-risk factor in MM and targeting the FABP family may hold great promise as a new treatment avenue for MM patients.

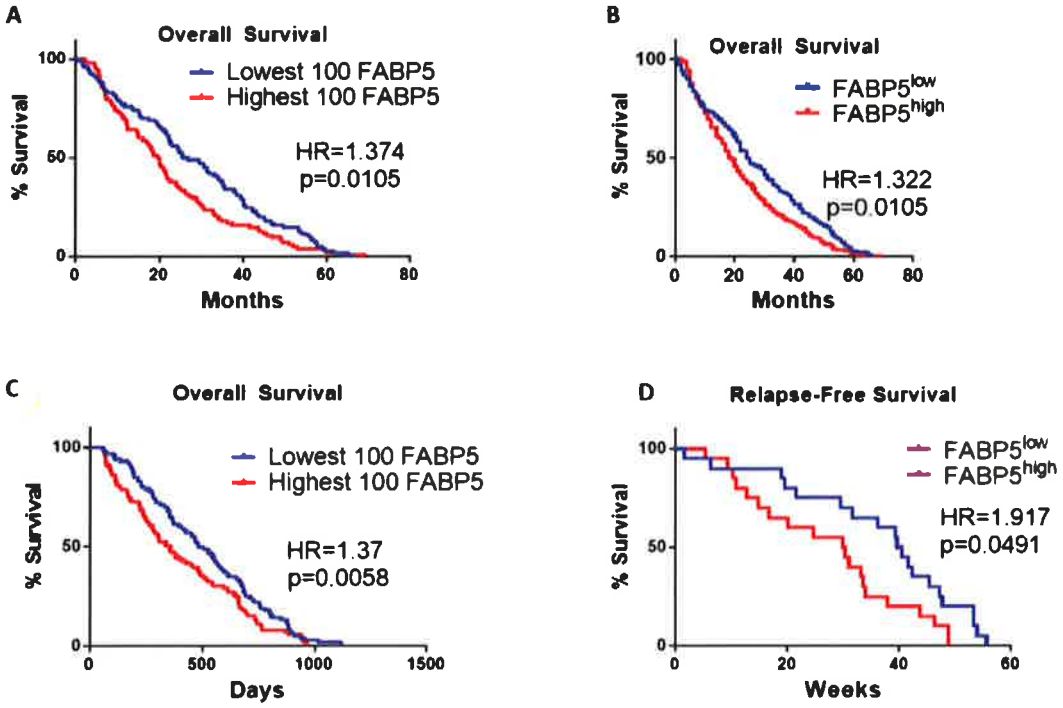
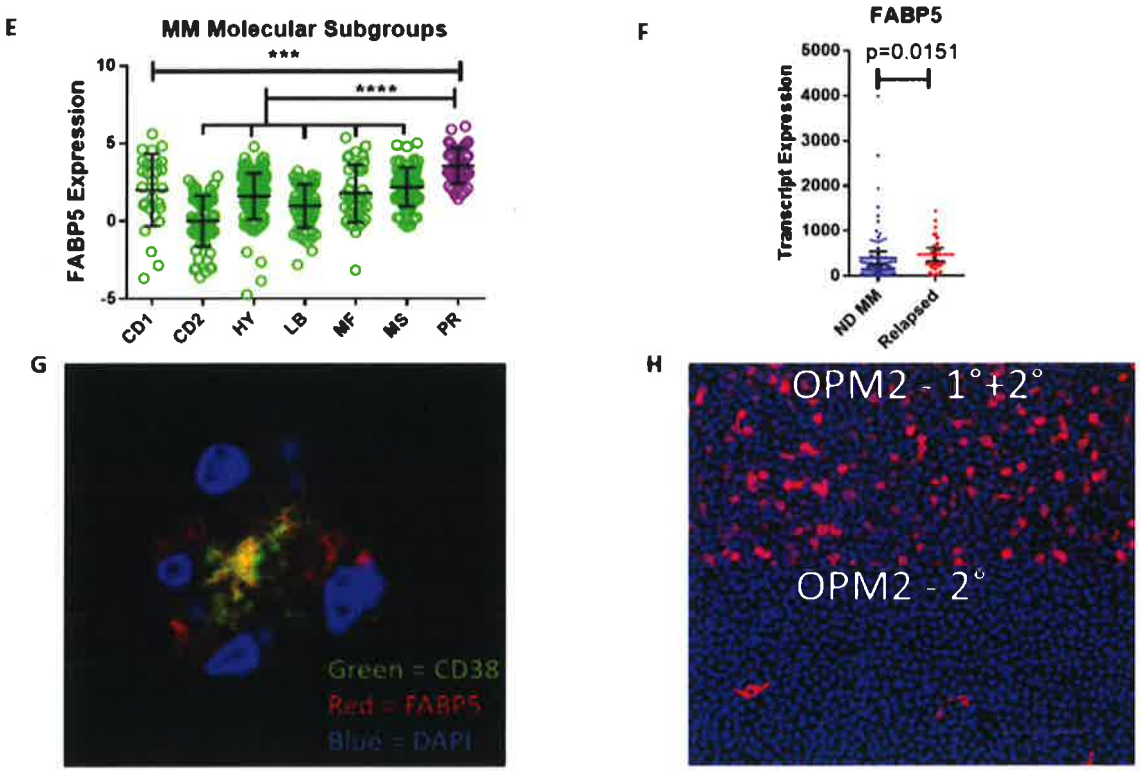


Figure 2. FABP5 expression levels corresponds to worse clinical outcomes in MM. A, B) Kaplan–Meier analysis of overall survival of myeloma patient groups in Zhan et al dataset stratified as top (n=100) or bottom (n=100) *FABP5*, or high (n=207) and low (n=207) *FABP5*. C) Kaplan–Meier analysis of overall survival of high (n=100) and low (n=100) *FABP5* expression in MM patient in Mulligan et al. dataset. D) Kaplan–Meier analysis of relapse-free survival of high (n = 20) and low (n = 20) *FABP5* expression in MM patient in Carrasco et al. dataset.

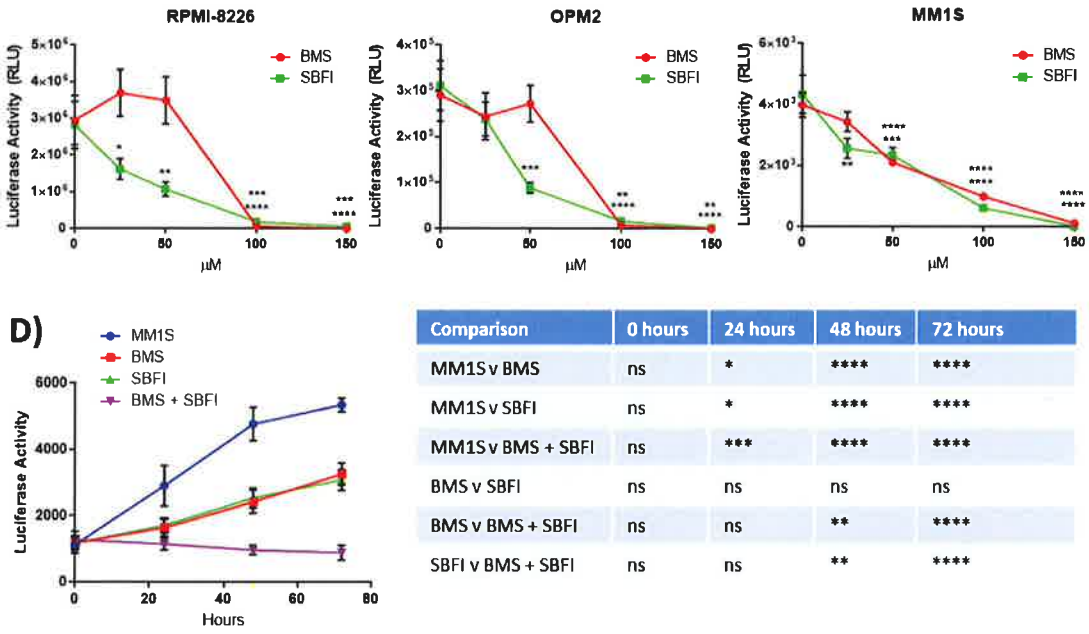


Continued Figure 2. FABP5 expression levels correspond to worse clinical outcomes in MM. E) Molecular subtypes of MM cells were analyzed for FABP5 expression and significance between all groups and the highly aggressive subtype (PR, proliferation) was observed using a One-way ANOVA with Dunnett's multiple comparison testing. F) Data is shown from Chng from newly-diagnosed (ND) (n=73) and relapsed MM patients (n=28) as mean with 95% confidence interval (CI), with statistical analysis performed using a Mann Whitney test. G) Myeloma cells from a patient stained with CD38 (green), FABP5 (red) and DAPI (blue). H) OPM2 cells stained with FABP5 (red) and DAPI (blue). Controls show the cells stained with the secondary antibody alone (2°).

Fatty acid binding protein inhibition impairs MM cell growth and induces apoptosis in myeloma cells in a dose-dependent manner.

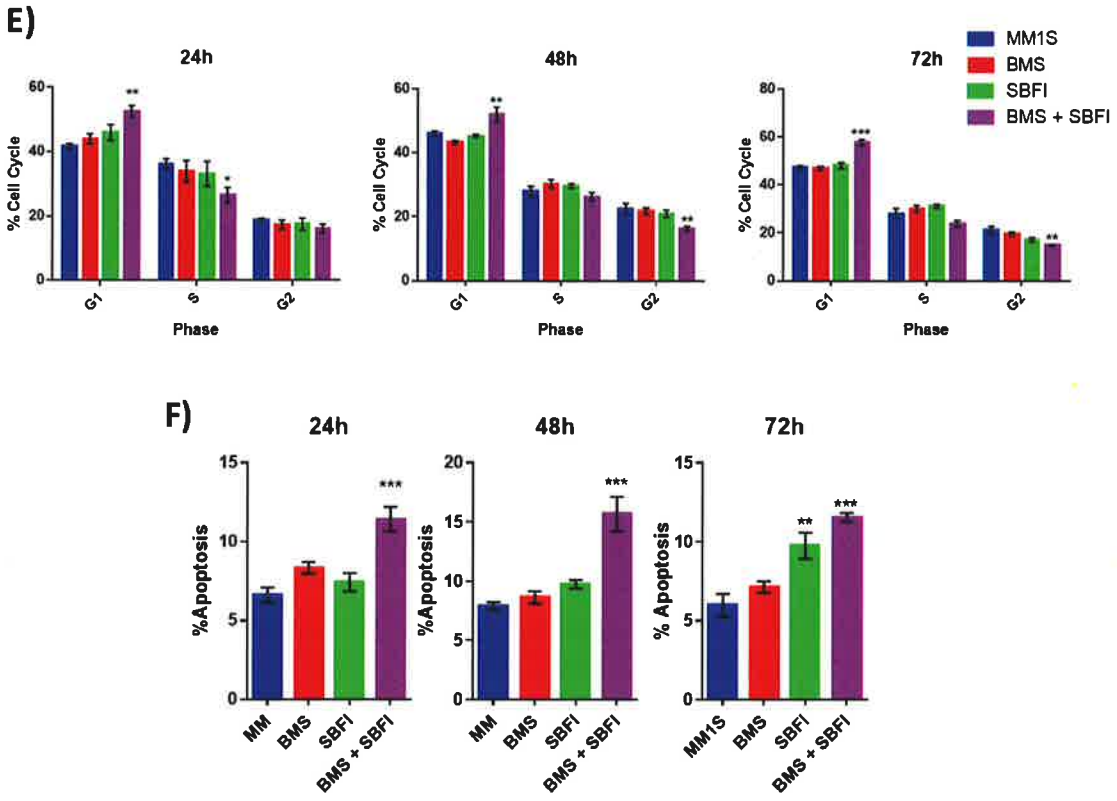
Next, we investigated the impact of FABP inhibition in several human MM cell lines using two well-known FABP inhibitors, BMS309403 (BMS) and SBFI-26 (SBFI). These inhibitors have been used to inhibit FABP 3, 4, 5, and 7, in several other publications, suggesting that these inhibitors could be targeting other FABPs within MM cells (Al-Jameel et al., 2017; Huang et al., 2017; Laouirem et al., 2019). However, according to RNAseq data from Fairfield et al. (Suppl. Table 1A), FABP3, 4, and 7 are expressed at a much lower level than FABP5. A 72-hour dose curve of BMS and SBFI demonstrated a decrease in RPMI8226, OPM2, and MM1S cell numbers in a dose-dependent manner. Both RPMI8226 and OPM2 had little to no luciferase activity by 100 μM, and 150 μM in the MM1S (Fig. 3A-C). To determine if this effect was specific to MM cells, human mesenchymal stem cells were exposed to similar doses, revealing no significant decrease in total ATP activity, a surrogate marker for cell number, unless at the high dose of 150 μM (data not shown). Next, we investigated how the inhibitors reduced cell numbers over time. BLI revealed that within 72 hours, the vehicle treated cells tripled in number, as expected. Single treatment of either inhibitor significantly reduced cell number compared to the control at the 72 hour time point (61%, BMS, and 57%, SBFI compared to the vehicle). Interestingly, the combination treatment significantly stunted growth (17% decrease in growth compared to day 0 seeding density) (Fig. 3D). Next, we investigated cell cycle and apoptosis using flow-based analysis to determine the influence of the FABP inhibitors. As early as 24 hours, we saw an increase in G1/G0 with the single inhibitors, with a significant increase with combination treatment. The increase in G1/G0

persisted throughout the 72 hour time course. Additionally, we saw a significant decrease in G2/M with both single inhibitors and the greatest reduction with the combination (Fig. 3E), overall suggesting a negative impact on cell cycle progression. In terms of apoptosis, we saw a significant effect of the combination as early as 24 hours and this persisted throughout the duration of the time course. By 72 hours, SBFI and the combination had significantly more apoptosis than the vehicle (Fig. 3F). These data suggest inhibition of FABPs significantly impairs cell cycle progression and induces apoptosis in human MM cells.



Comparison	0 hours	24 hours	48 hours	72 hours
MM1S v BMS	ns	*	****	****
MM1S v SBFI	ns	*	****	****
MM1S v BMS + SBFI	ns	***	****	****
BMS v SBFI	ns	ns	ns	ns
BMS v BMS + SBFI	ns	ns	**	****
SBFI v BMS + SBFI	ns	ns	**	****

Figure 3. FABP inhibition with SBFI or BMS significantly impairs cell growth, cell cycle and induced apoptosis in MM cell lines. A-C) RPMI8226, OPM2, and MM1S cells respond to SBFI or BMS in a dose dependent manner within 72 hours. D) MM1S cells have significantly reduced cell growth over 72 hours with 50 μM BMS, 50 μM SBFI or combination treatment of both at 50 μM doses.



Continued Figure 3. FABP inhibition with SBFI or BMS significantly impairs cell growth, cell cycle and induced apoptosis in MM cell lines. E) Cell cycle and F) apoptosis is negatively impacted with 50 μM BMS, 50 μM SBFI or combination treatment of both at 50 μM in MM1S cells over a 72 hour time course.

BMS, SBFI or the combination significantly decrease tumor burden and improve survival in a multiple myeloma xenograft mouse model.

To confirm our *in vitro* findings of myeloma growth inhibition with FABP inhibitors, we moved *in vivo* to treat MM1S inoculated in 8-week-old, female Scid-beige mice. One day post-inoculation, treatment began with either BMS, SBFI or the combination three times weekly, i.p (Fig. 4A). Either single treatment or the combination did not significantly influence the weight of the mice compared to the vehicle (Fig. 4B). To monitor tumor progression, mice were subjected to bioluminescence imaging twice weekly. A significant difference in tumor burden was detected as early as day 21 in the BMS, SBFI and combination groups compared to the control. This trend in decreased tumor burden continued throughout the duration of the study for all treatment groups. At day 28, we saw significantly less tumor burden in the BMS treatment compared to either the SBFI or the combination, but this was not reflected in the survival of the mice (Fig 4C-E). In fact, mice that received BMS, SBFI or the combination had significantly longer survival than the vehicle mice, which highlights the great promise of targeting the FABPs as a potential treatment. We did not observe any negative side effects or signs of sickness from the FABP inhibitors in these mice. Overall, single or combination treatment with BMS and SBFI significantly extended the survival of the mice and reduced tumor burden in myeloma inoculated mice.

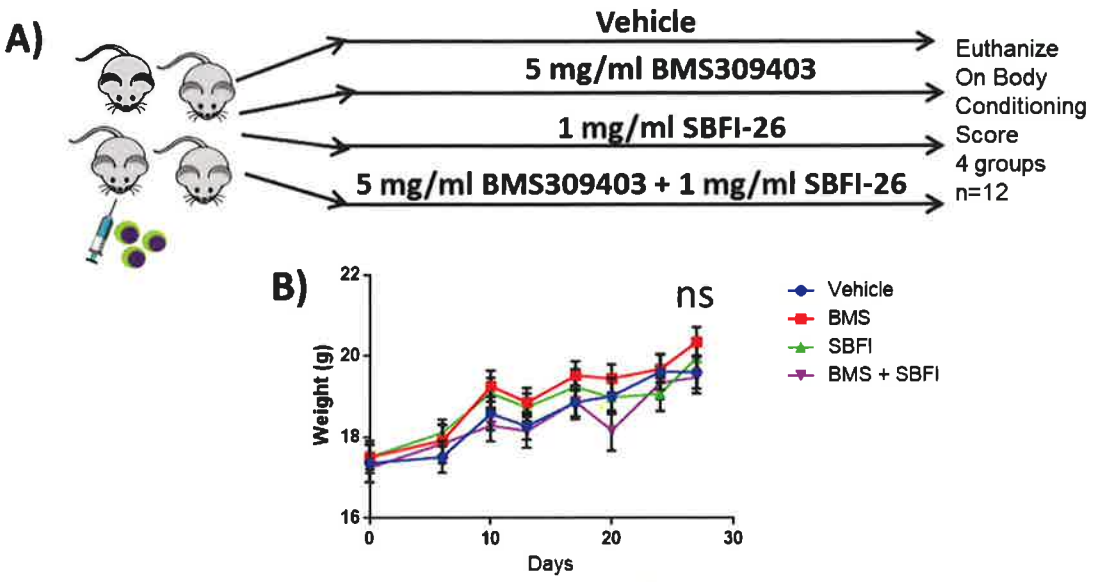
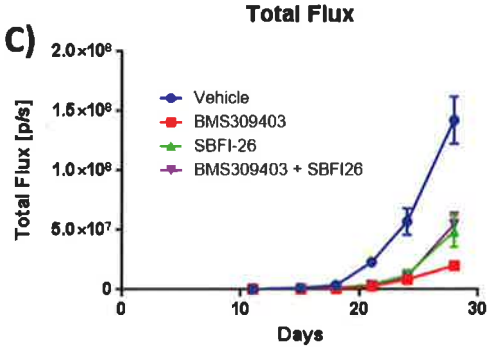
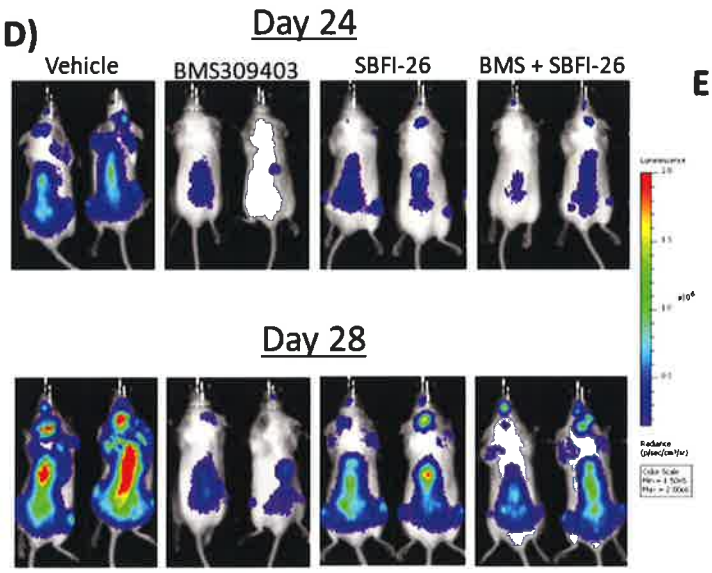
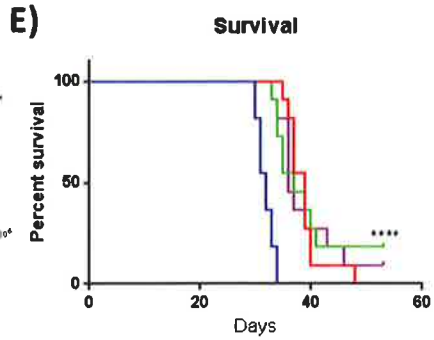


Figure 4. FABP inhibition with SBFI or BMS significantly reduces tumor burden in the MM1S xenograft model. A, B) Mice treated with the 1 mg/kg SBFI, 5 mg/kg BMS or combination treatment have no negative effect on weight. C, E) Bioluminescent imaging revealed a decrease in tumor bearing mice treated with FABP inhibitors compared to vehicle treated mice.



Day 24		Day 28	
Vehicle vs BMS	****	Vehicle vs BMS	****
Vehicle vs SBFI-26	****	Vehicle vs SBFI-26	****
Vehicle vs BMS + SBFI-26	****	Vehicle vs BMS + SBFI-26	****
BMS vs SBFI-26	ns	BMS vs SBFI-26	**
BMS vs BMS + SBFI-26	ns	BMS vs BMS + SBFI-26	***
SBFI-26 vs BMS + SBFI-26	ns	SBFI-26 vs BMS + SBFI-26	ns





Continued Figure 4. FABP inhibition with SBFI or BMS significantly reduces tumor burden in the MM1S xenograft model. C-E) Bioluminescent imaging revealed a decrease in tumor bearing mice treated with FABP inhibitors compared to vehicle treated mice.

Inhibition of fatty acid binding proteins increases the efficacy of dexamethasone *in vitro* and reduces MM cell number in the presence of bone marrow adipocyte condition media.

Since we observed concurrent dexamethasone resistance and FABP4 expression in MM cells cultured in BMAd CM, and that inhibition of FABPs impacted MM cell growth, we hypothesized that inhibiting FABPs would resensitize MM cells to dexamethasone in BMAd CM conditions. In basal media, dexamethasone in combination with the single inhibitors significantly reduced cell number compared to all single treatments (~20% survival of the control). However, the greatest reduction to cell number was the combination treatment of dexamethasone, BMS and SBFI, resulting in ~5% survival (Fig. 5A). In BMAd CM conditions, there was no reduction in cell number with dexamethasone, reaffirming resistance. Single inhibitors reduced MM cell numbers compared to the BMAd CM control (BMS 33%, and SBFI 41%). In combination, BMS and SBFI significantly reduced cell number compared to the BMAd CM control (BMS + SBFI 12%) and the BMAd CM single inhibitor treatments (21% compared to BMS, and 29% compared to SBFI). Surprisingly in BMAd CM conditions, dexamethasone combined with either single inhibitors or the combination of inhibitors did not resensitize the MM cells to dexamethasone. Combination treatment of dexamethasone and BMS or SBFI had similar luciferase activity to the single inhibitors. The triple treatment had the same luciferase activity as the BMS and SBFI combination (Fig. 5B). Consistent with these findings, dexamethasone induced a 3-fold increase in apoptosis in basal conditions, but there was no increase in apoptosis with the single inhibitors (Fig. 5C). Dexamethasone co-treatment with either inhibitor resulted in significant apoptosis

compared to the control or dexamethasone alone. The combination of both inhibitors did not increase apoptosis; however, a combination of BMS, SBFI and dexamethasone triggered a 7-fold increase in apoptosis compared to the control. Importantly, dual inhibition with dexamethasone treatment significantly increased MM cell apoptosis compared to dexamethasone alone, or the single inhibitors and dexamethasone (Fig. 5C). In BMAd CM conditions, dexamethasone alone did not increase apoptosis relative to control, nor did the single inhibitors. Interestingly, triple treatment induced significantly more apoptosis compared to the control (1.8-fold), dexamethasone alone, BMS alone, or BMS and dexamethasone combination. In comparison, there was no significance between BMS alone, SBFI and dexamethasone, or BMS and SBFI (Fig. 5D, Suppl. Table 2). While FABP inhibition did not reverse drug resistance, it still reduced cell number and induced apoptosis in a rich, pro-myeloma environment.

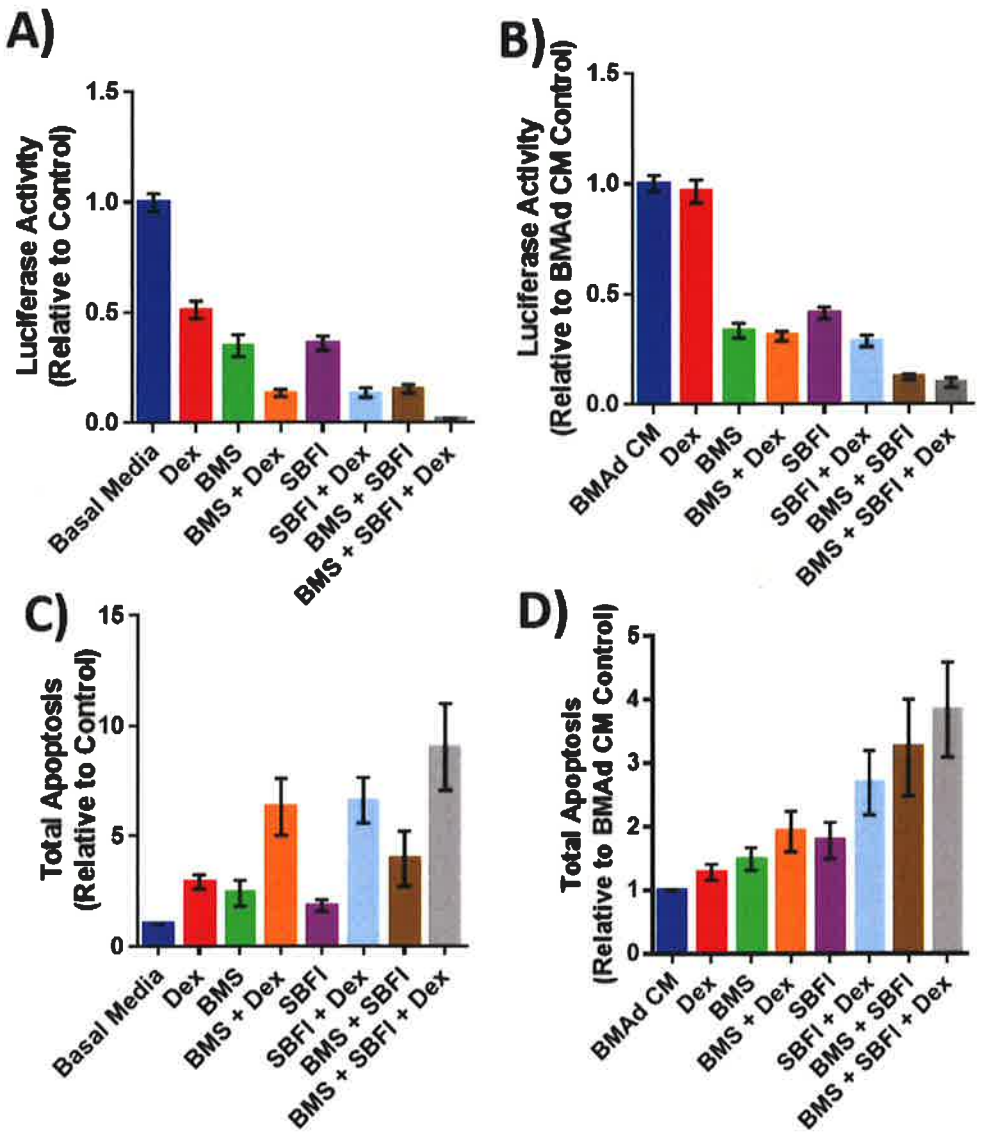
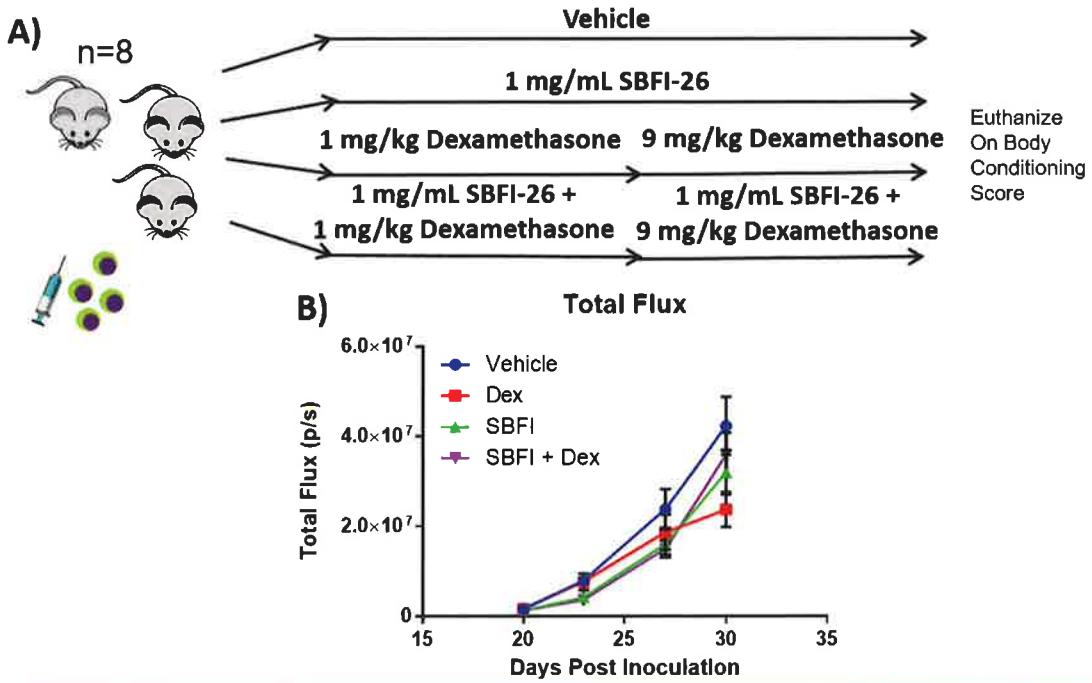


Figure 5. BMAd CM induced dexamethasone resistance in MM1S cells is not reverse with FABP inhibition. A) MM1S cells treated with 80 μM dex, 50 μM BMS, 50 μM SBFI, or the combination reduces cell number. B) BMAd CM induced dex resistance, but BMS and SBFI treatment reduces cell number. C) Dex, BMS, SBFI or the combinations induce apoptosis after 72 hour treatment in MM1S cells. D) BMAd CM induces dex resistance, but BMS, SBFI and the combinations induce apoptosis after 72 hour treatment in MM1S cells.

***In vivo* combination treatment of FABP inhibitor with dexamethasone does not reduce tumor burden.**

To test our final hypothesis that an FABP inhibitor would synergize with dexamethasone *in vivo*, we combined SBFI and dexamethasone in the Scid-beige MM1S mouse model. The treatment schedule was that either the vehicle, 1 mg/kg SBFI, 1 mg/kg dexamethasone, or the combination was administered i.p for the first three weeks of treatment. After, seeing no combined effect with this low dose of dexamethasone, the dexamethasone was increased to 9 mg/kg for the duration of the study (Fig. 6A). There were no negative side effects of the SBFI or dexamethasone treatments on weight or behavior of the mice (Suppl. Fig. 3A). BLI revealed that 1 mg/kg dexamethasone did not significantly reduce tumor burden within three weeks, but when dexamethasone was increased to 9 mg/kg, tumor burden was significantly reduced (Fig. 6B-C). Interestingly, the "SBFI only" group had a significant negative effect at day 23, which set a trend for less tumor burden throughout the study (Suppl. Fig. 3B). Surprisingly, combination treatment did not significantly reduce tumor burden compared to the vehicle, SBFI alone, or dexamethasone alone. Overall, these data suggest that while dexamethasone and SBFI may not synergize *in vivo,* SBFI is a powerful single inhibitor.



Day 20		Day 23		Day 27		Day 30	
Vehicle vs Dex	ns	Vehicle vs Dex	ns	Vehicle vs Dex	ns	Vehicle vs Dex	***
Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns	Vehicle vs SBFI-26	ns
Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns	Vehicle vs Dex + SBFI-26	ns
Dex vs SBFI-26	ns	Dex vs SBFI-26	ns	Dex vs SBFI-26	ns	Dex vs SBFI-26	ns
Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	ns	Dex vs Dex + SBFI-26	*
SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns	SBFI-26 vs Dex + SBFI-26	ns

Figure 6. SBFI is as potent as a single agent compared to combination with dexamethasone in MM1S xenograft study. A-B) MM1S xenograft mice treated with 1 mg/kg SBFI, 1 mg/kg and 9 mg/kg dex, or the combination have various responses with tumor burden.

DISCUSSION

In this thesis, I describe a series of studies where we examined the relationship between BMAd, myeloma cells and FABPs. We also studied how FABPs and BMAd can alter myeloma cell growth, apoptosis, proliferation, and response to a commonly prescribed chemotherapeutic agent, dexamethasone. We found that in MM1S and OPM2 cells, cell numbers were increased in BMAd derived conditioned media. Additionally, MM1S cells were resistant to dexamethasone in BMAd CM as shown by a rescue in their cell number, a decrease in their apoptotic response, a rescue of their cell cycle progression, and restoration of their Ki67 potential. BMAd release a high volume of FABP4, and exposure to BMAd CM elicits an increase in internal FABP4 in MM1S cells, supporting recent findings (Shu, 2020). Additionally, we observed that the fatty acid binding protein family is important to MM cell growth using 3 cell lines that represent different type of MM harboring different genetic abnormalities (RPMI8226, MM1S and OPM2 cell lines). Basally, single inhibitors of FABP4 and 5 significantly impaired cell growth over time, and this is consistent even in the presence of tumor-supportive BMAd CM. In addition, when the single inhibitors were used in combination with dexamethasone, there was a significant decrease in cell number and significantly increased apoptosis compared to dexamethasone treatment alone in basal conditions. Importantly, combination treatment of BMS, SBFI and dexamethasone resulted in the greatest reduction in cell number and significantly higher apoptosis compared to any other treatment, resulting in an almost 7-fold increase compared to the control in basal conditions. While FABP inhibition did not reverse drug resistance in BMAd CM, combining the duel inhibitors resulted in a significant increase in apoptosis and significant reduction in cell number. In summary,

these data suggest that duel inhibition of BMS and SBFI does not resensitize MM cells to dexamethasone in BMAd CM, and supports previous studies that additional adipokines are likely involved in BMAd-induced drug resistance (H. Liu et al., 2019; Z. Liu et al., 2015). A need for future studies in myeloma dexamethasone drug resistance are needed to elucidate other molecules in BMAd CM alleviating MM cells from dexamethasone-induced apoptosis (M. L. Farrell & Reagan, 2018).

Overall, targeting the FABP family appears to be a promising new target in myeloma, which could prove to be relevant in essentially all other forms of cancer. While targeting FABPs does not reverse dexamethasone resistance, targeting FABP5 looks to be clinically and translationally promising in multiple myeloma. FABP3, FABP4, FABP5, and FABP6 are expressed in three common myeloma lines, with FABP5 being the highest expressed. Clinically, high FABP5 expression correlates with poor outcomes and is significantly higher in relapsed patients. Targeting the family of FABPs with the pharmacological pan inhibitors, SBFI-26 and BMS309403, impaired myeloma growth and induced a level of apoptosis *in vitro.* Inhibition of FABPs *in vivo* significantly reduced tumor burden and extended the life span of mice. While our dexamethasone-SBFI *in vivo* experiment did not demonstrate synergy, it is possible that higher doses than we used herein could have better effects. Our data suggest that targeting FABPs could be a beneficial and important avenue to treat myeloma or other cancer patients (M. Farrell, Fairfield, D'Amico, Murphy, & Reagan, 2020).

In future studies, we plan to broaden the scope of combination treatments to extend to other anti-myeloma therapies both in terms of drug resistance and combination of FABP inhibitors. We would plan to interrogate the *in vitro* effects of BMAds and FABPs on MM resistance to proteasome inhibitors and/or immunomodulatory imide drugs, and determine the mechanisms driving this type of resistance. *In vivo,* we plan to use mouse models that have high BMAT either due to irradiation or diet, and test if MM cells are resistant to other agents beyond dexamethasone. This year, I also published as first author a manuscript showing that BM adiposity is reduced with anti-sclerostin antibody treatment, suggesting that anti-sclerostin antibody could be used in combination with dexamethasone to elucidate if MM cells respond better to dexamethasone when the microenvironment is depleted of BMAd (M. Farrell, Fairfield, Costa, et al., 2020). We are also interested in using a commonly used antibiotic, Levofloxacin, to treat myeloma burdened mice to reduce tumor growth. Levofloxacin has been showed to inhibit FABP4 activity and is currently used in the clinic for treatments in other cancers (Mukherjee et al., 2020).

Myeloma is currently incurable and the median survival is only five years, highlighting that novel new treatments are needed to expand the quality and duration of life. Our data illuminate that FABP5 is a novel therapeutic target and our findings demand more investigation into its role in myeloma due to its potential of curing, or increasing life expectancy for patients with this deadly disease.

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REFERENCES

Al-Jameel, W., Gou, X., Forootan, S. S., Al Fayi, M. S., Rudland, P. S., Forootan, F. S., … Ke, Y. (2017). Inhibitor SBFI26 suppresses the malignant progression of castration-resistant PC3-M cells by competitively binding to oncogenic FABP5. *Oncotarget*, *8*(19), 31041–31056. https://doi.org/10.18632/oncotarget.16055

Al-Jameel, W., Gou, X., Jin, X., Zhang, J., Wei, Q., Ai, J., … Ke, Y. (2019). Inactivated FABP5 suppresses malignant progression of prostate cancer cells by inhibiting the activation of nuclear fatty acid receptor PPARγ. *Genes and Cancer*, *10*(3–4), 80–96. https://doi.org/10.18632/oncoscience.192

Alexander, D. D., Mink, P. J., Adami, H. O., Cole, P., Mandel, J. S., Oken, M. M., & Trichopoulos, D. (2007, January 1). Multiple myeloma: A review of the epidemiologic literature. *International Journal of Cancer*, Vol. 120, pp. 40–61. https://doi.org/10.1002/ijc.22718

Bornstein, S., Moschetta, M., Kawano, Y., Sacco, A., Huynh, D., Brooks, D., … Reagan,

M. R. (2017). Metformin Affects Cortical Bone Mass and Marrow Adiposity in Diet-Induced Obesity in Male Mice. *Endocrinology*, *158*(10), 3369–3385. https://doi.org/10.1210/en.2017-00299

Bullwinkle, E. M., Parker, M. D., Bonan, N. F., Falkenberg, L. G., Davison, S. P., & Decicco-Skinner, K. L. (2016). *Adipocytes contribute to the growth and progression of multiple myeloma: Unraveling obesity related differences in adipocyte signaling*. https://doi.org/10.1016/j.canlet.2016.06.010

Carbonetti, G., Wilpshaar, T., Kroonen, J., Studholme, K., Converso, C., d'Oelsnitz, S., & Kaczocha, M. (2019). FABP5 coordinates lipid signaling that promotes prostate cancer metastasis. *Scientific Reports*, *9*(1), 18944. https://doi.org/10.1038/s41598-019-55418-x

Carrasco, D. R., Tonon, G., Huang, Y., Zhang, Y., Sinha, R., Feng, B., … DePinho, R. A. (2006). High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell*, *9*(4), 313–325. https://doi.org/10.1016/j.ccr.2006.03.019

Chng, W. J., Kumar, S., Vanwier, S., Ahmann, G., Price-Troska, T., Henderson, K., … Fonseca, R. (2007). Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. *Cancer Research*, *67*(7), 2982–2989. https://doi.org/10.1158/0008-5472.CAN-06-4046

Fairfield, H., Dudakovic, A., Khatib, C. M., Farrell, M., Costa, S., Falank, C., … Reagan, M. R. (2020). Myeloma-modified adipocytes exhibit metabolic dysfunction and a senescence-associated secretory phenotype (SASP). *Cancer Research, In Press*. https://doi.org/10.1158/0008-5472.CAN-20-1088

Fairfield, H., Falank, C., Avery, L., & Reagan, M. R. (2016). Multiple myeloma in the marrow: pathogenesis and treatments. *Annals of the New York Academy of Sciences*, *1364*(1), 32–51. https://doi.org/10.1111/nyas.13038

Fairfield, H., Falank, C., Farrell, M., Vary, C., Boucher, J. M., Driscoll, H., … Reagan, M. R. (2019). Development of a 3D bone marrow adipose tissue model. *Bone*, Vol. 118, pp. 77–88. https://doi.org/10.1016/j.bone.2018.01.023

Falank, C., Fairfield, H., Farrell, M., & Reagan, M. R. (2017). New Bone Cell Type Identified As Driver of Drug Resistance in Multiple Myeloma: The Bone Marrow Adipocyte. *Blood*, *130*(Suppl 1), 122.

Farrell, M., Fairfield, H., Costa, S., D'Amico, A., Falank, C., Brooks, D. J., & Reagan, M. R. (2020). Sclerostin-Neutralizing Antibody Treatment Rescues Negative Effects of Rosiglitazone on Mouse Bone Parameters . *Journal of Bone and Mineral Research*. https://doi.org/10.1002/jbmr.4170

Farrell, M., Fairfield, H., D'Amico, A., Murphy, C. S., & Reagan, M. R. (2020). Targeting Bone Marrow Adipose Tissue and the FABP Family Increases Efficacy of Dexamethasone in Multiple Myeloma. Retrieved December 14, 2020, from 62nd ASH Annual Meeting and Exposition website: https://annualmeeting.hematology.org/posters/25578560/Targeting-Bone-Marrow-Adipose-Tissue-and-the-FABP-Family-Increases-Efficacy-of-Dexamethasone-in-Multiple-Myeloma

Farrell, M. L., & Reagan, M. R. (2018). Soluble and cell-cell-mediated drivers of proteasome inhibitor resistance in multiple myeloma. *Frontiers in Endocrinology*, *9*(MAY). https://doi.org/10.3389/fendo.2018.00218

Guaita-Esteruelas, S., Bosquet, A., Saavedra, P., Gumà, J., Girona, J., Lam, E. W.-F., … Masana, L. (2016). Exogenous FABP4 increases breast cancer cell proliferation and activates the expression of fatty acid transport proteins. *Molecular Carcinogenesis*. https://doi.org/10.1002/mc.22485

Hardaway, A. L., Herroon, M. K., Rajagurubandara, E., & Podgorski, I. (2014). Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases. *Cancer Metastasis Reviews*, *33*(2–3), 527–543. https://doi.org/10.1007/s10555-013-9484-y

Herroon, M., Rajagurubandara, E., Hardaway, A. L., Powell, K., Turchick, A., Feldmann, D., & Podgorski, I. (2013). Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms. *Oncotarget*, *4*(11), 2108–2123. https://doi.org/10.18632/oncotarget.1482

Huang, M., Narita, S., Inoue, T., Koizumi, A., Saito, M., Tsuruta, H., … Habuchi, T. (2017). Fatty acid binding protein 4 enhances prostate cancer progression by upregulating matrix metalloproteinases and stromal cell cytokine production. *Oncotarget*, *8*(67), 111780–111794. https://doi.org/10.18632/oncotarget.22908

Landgren, O., & Iskander, K. (2017). Modern multiple myeloma therapy: deep, sustained treatment response and good clinical outcomes. *Journal of Internal Medicine*, *281*(4), 365–382. https://doi.org/10.1111/joim.12590

Laouirem, S., Sannier, A., Norkowski, E., Cauchy, F., Doblas, S., Rautou, P. E., … Paradis, V. (2019). Endothelial fatty liver binding protein 4: a new targetable mediator in hepatocellular carcinoma related to metabolic syndrome. *Oncogene*, *38*(16), 3033–3046. https://doi.org/10.1038/s41388-018-0597-1

Lee, M. Y., Li, H., Xiao, Y., Zhou, Z., Xu, A., & Vanhoutte, P. M. (2011). Chronic administration of BMS309403 improves endothelial function in apolipoprotein E-deficient mice and in cultured human endothelial cells Keywords A-FABP; endothelium-dependent relaxation; eNOS; endothelial dysfunction; nitric oxide; atherosclerosis. *1564 British Journal of Pharmacology*, *162*, 1564–1576. https://doi.org/10.1111/j.1476-5381.2010.01158.x

Liu, H., He, J., Koh, S. P., Zhong, Y., Liu, Z., Wang, Z., … Yang, J. (2019). Reprogrammed marrow adipocytes contribute to myeloma-induced bone disease. *Science Translational Medicine*, *11*(494). https://doi.org/10.1126/scitranslmed.aau9087

Liu, R. Z., Graham, K., Glubrecht, D. D., Germain, D. R., Mackey, J. R., & Godbout, R. (2011). Association of FABP5 expression with poor survival in triple-negative breast cancer: Implication for retinoic acid therapy. *American Journal of Pathology*, *178*(3), 997–1008. https://doi.org/10.1016/j.ajpath.2010.11.075

Liu, Z., Xu, J., He, J., Liu, H., Lin, P., Wan, X., … Yang, J. (2015). Mature adipocytes in bone marrow protect myeloma cells against chemotherapy through autophagy activation. *Oncotarget*, *6*(33), 34329–34341. https://doi.org/10.18632/oncotarget.6020

Lv, Q., Wang, G., Zhang, Y., Han, X., Li, H., Le, W., … Ding, Q. (2019). FABP5 regulates the proliferation of clear cell renal cell carcinoma cells via the PI3K/AKT signaling pathway. *International Journal of Oncology*, *54*(4), 1221–1232. https://doi.org/10.3892/ijo.2019.4721

Ma, R., Wang, L., Yuan, F., Wang, S., Liu, Y., Fan, T., & Wang, F. (2018). FABP7

promotes cell proliferation and survival in colon cancer through MEK/ERK signaling pathway. *Biomedicine and Pharmacotherapy*, *108*, 119–129. https://doi.org/10.1016/j.biopha.2018.08.038

Marinac, C. R., Suppan, C. A., Giovannucci, E., Song, M., Kvaerner, A. S., Townsend, M. K., … Birmann, B. M. (2019). Elucidating Under-Studied Aspects of the Link Between Obesity and Multiple Myeloma: Weight Pattern, Body Shape Trajectory, and Body Fat Distribution. *JNCI Cancer Spectrum*, *3*(3). https://doi.org/10.1093/jncics/pkz044

Morris, E. V, Suchacki, K. J., Hocking, J., Cartwright, R., Sowman, A., Gamez, B., … Edwards, C. M. (2020). Myeloma Cells Down-Regulate Adiponectin in Bone Marrow Adipocytes Via TNF-Alpha. *Journal of Bone and Mineral Research*, jbmr.3951. https://doi.org/10.1002/jbmr.3951

Mukherjee, A., Chiang, C. Y., Daifotis, H. A., Nieman, K. M., Fahrmann, J. F., Lastra, R. R., … Lengyel, E. (2020). Adipocyte-induced FABP4 expression in ovarian cancer cells promotes metastasis and mediates carboplatin resistance. *Cancer Research*, *80*(8), 1748–1761. https://doi.org/10.1158/0008-5472.CAN-19-1999

Mulligan, G., Mitsiades, C., Bryant, B., Zhan, F., Chng, W. J., Roels, S., … Anderson, K. C. (2007). Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. *Blood*, *109*(8), 3177–3188. https://doi.org/10.1182/blood-2006-09-044974

Myeloma — Cancer Stat Facts. (n.d.). Retrieved September 30, 2020, from https://seer.cancer.gov/statfacts/html/mulmy.html

Natoni, A., Farrell, M. L., Harris, S., Falank, C., Kirkham-McCarthy, L., Macauley, M. S., … O'Dwyer, M. (2020). Sialyltransferase inhibition leads to inhibition of tumor cell interactions with E-selectin, VCAM1, and MADCAM1, and improves sur vival in a human multiple myeloma mouse model. *Haematologica*, *105*(2), 457–467. https://doi.org/10.3324/haematol.2018.212266

Rajkumar, S. V. (2020). Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. *American Journal of Hematology*, *95*(5), 548–567. https://doi.org/10.1002/ajh.25791

Reagan, M. R., Liaw, L., Rosen, C. J., & Ghobrial, I. M. (2015, June 1). Dynamic interplay between bone and multiple myeloma: Emerging roles of the osteoblast. *Bone*, Vol. 75, pp. 161–169. https://doi.org/10.1016/j.bone.2015.02.021

Reagan, M. R., & Rosen, C. J. (2015). Navigating the bone marrow niche: translational insights and cancer-driven dysfunction. *Nature Reviews Rheumatology*. https://doi.org/10.1038/nrrheum.2015.160

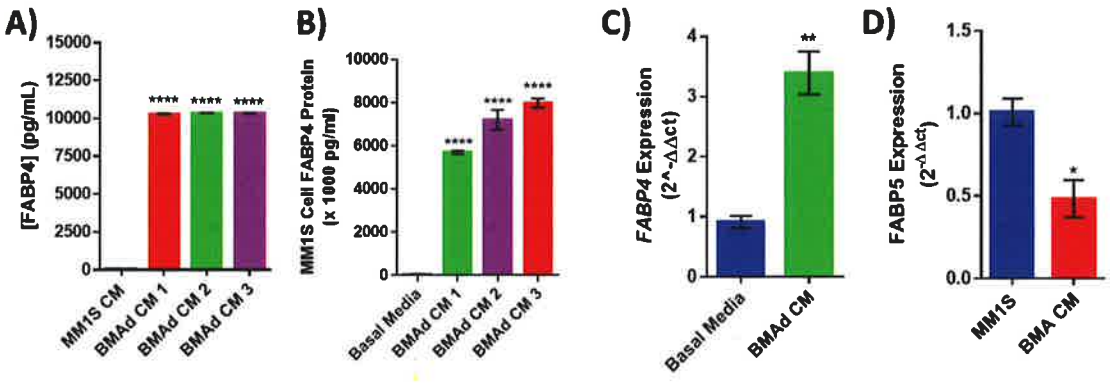
Shafat, M. S., Oellerich, T., Mohr, S., Robinson, S. D., Edwards, D. R., Marlein, C. R., … Rushworth, S. A. (2017). Leukemic blasts program bone marrow adipocytes to generate a protumoral microenvironment. *Blood*, *129*(10), 1320–1332. https://doi.org/10.1182/blood-2016-08-734798

Shu, L. (2020). Adipocyte Fatty Acid Binding Protein Promotes Multiple Myeloma through Regulating Bone Marrow Microenvironment . Retrieved December 14, 2020, from 62nd ASH Annual Meeting and Exposition website: https://annualmeeting.hematology.org/posters/25578571/Adipocyte-Fatty-Acid-Binding-Protein-Promotes-Multiple-Myeloma-through-Regulating-Bone-Marrow-Microenvironment

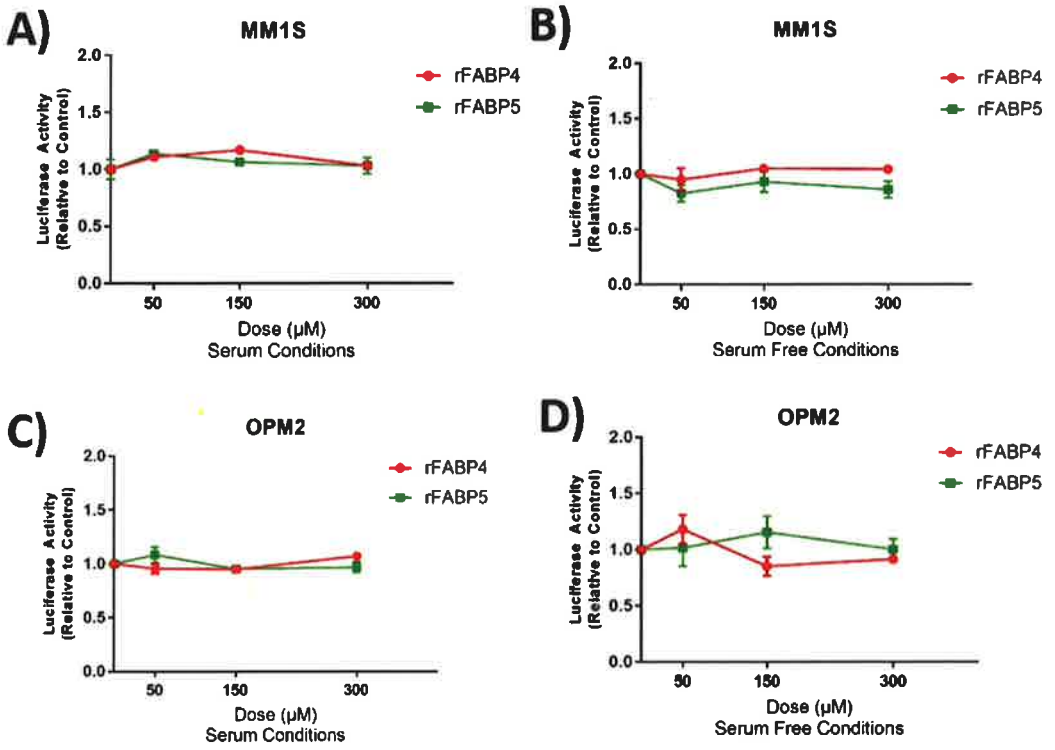
Trotter, T. N., Gibson, J. T., Sherpa, T. L., Gowda, P. S., Peker, D., & Yang, Y. (2016). Adipocyte-Lineage Cells Support Growth and Dissemination of Multiple Myeloma in Bone. *American Journal of Pathology*, *186*(11), 3054–3063. https://doi.org/10.1016/j.ajpath.2016.07.012

Veldhuis-Vlug, A. G., & Rosen, C. J. (2018, February). Clinical implications of bone marrow adiposity. *Journal of Internal Medicine*, Vol. 283, pp. 121–139. https://doi.org/10.1111/joim.12718

Zhan, F., Huang, Y., Colla, S., Stewart, J. P., Hanamura, I., Gupta, S., … Shaughnessy, J. D. (2006). The molecular classification of multiple myeloma. *Blood*, *108*(6), 2020–2028. https://doi.org/10.1182/blood-2005-11-013458



Supplemental Figure 1. FABP gene and protein expression levels are influenced by BMAd CM. A, B) FABP4 protein levels are higher in BMAd CM and in MM after BMAd CM treatment, as determined by ELISA. C) MM1S mRNA levels of FABP4 increase and D) FABP5 decrease after 72 hour treatment with



Supplemental Figure 2. Exogenously added recombinant FABP4 or 5 protein does not influence cell number. A, B) Exogenously added recombinant FABP4 or 5 protein did not influence MM1S cell number. C, D) Exogenously added recombinant FABP4 or 5 protein did not influence OPM2 cell number.

A)

GeneID (RPKM)	OPM2	MM.1S	RPMI-8226
FABP1	0	0	0
FABP2	0	0.019627	0
FABP3	1.727137	1.408361	1.710191
FABP4	1.54696	1.790967	1.946746
FABP5	114.043	98.42895	24.38133
FABP6	5.329104	1.272839	0.752652
FABP7	0	0	0
FABP9	0	0	0
FABP12	0	0	0

Supplemental Table 1. FABP5 is the highest expressed FABP in OPM2, RPMI8226 and MM1S cells. A) Basal expression levels of FABPs in OPM2, RPMI8226 and MM1S cells determined by RNAseq.

A)

Luciferase Activity - Comparison	Significance
Basal Media vs. Dex	****
Basal Media vs. BMS	****
Basal Media vs. SBFI	****
Basal Media vs. BMS + Dex	****
Basal Media vs. SBFI + Dex	****
Basal Media vs. BMS + SBFI	****
Basal Media vs. BMS + SBFI + Dex	****
Dex vs. BMS + Dex	****
Dex vs. SBFI + Dex	****
Dex vs. BMS + SBFI + Dex	****
BMS vs. BMS + Dex	***
BMS vs. BMS + SBFI	**
BMS vs. BMS + SBFI + Dex	****
SBFI vs. SBFI + Dex	***
SBFI vs. BMS + SBFI	***
SBFI vs. BMS + SBFI + Dex	****
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	ns

B)

Luciferase Activity - Comparison	Significance
BMAd CM vs. Dex	ns
BMAd CM vs. BMS	****
BMAd CM vs. SBFI	****
BMAd CM vs. BMS + Dex	****
BMAd CM vs. SBFI + Dex	****
BMAd CM vs. BMS + SBFI	****
BMAd CM vs. BMS + SBFI + Dex	****
Dex vs. BMS + Dex	****
Dex vs. SBFI + Dex	****
Dex vs. BMS + SBFI + Dex	****
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	***
BMS vs. BMS + SBFI + Dex	****
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	****
SBFI vs. BMS + SBFI + Dex	****
BMS + Dex vs. BMS + SBFI + Dex	***
SBFI + Dex vs. BMS + SBFI + Dex	**
BMS + SBFI vs. BMS + SBFI + Dex	ns

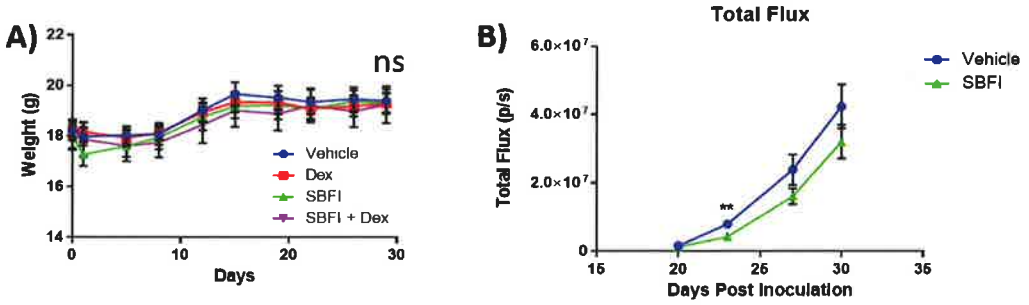
C)

Total Apoptosis- Comparison	Significance
Basal Media vs. Dex	ns
Basal Media vs. BMS	ns
Basal Media vs. SBFI	ns
Basal Media vs. BMS + Dex	*
Basal Media vs. SBFI + Dex	*
Basal Media vs. BMS + SBFI	ns
Basal Media vs. BMS + SBFI + Dex	***
Dex vs. BMS + Dex	ns
Dex vs. SBFI + Dex	ns
Dex vs. BMS + SBFI + Dex	**
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	ns
BMS vs. BMS + SBFI + Dex	**
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	ns
SBFI vs. BMS + SBFI + Dex	***
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	*

D)

Total Apoptosis- Comparison	Significance
BMAd CM vs. Dex	ns
BMAd CM vs. BMS	ns
BMAd CM vs. SBFI	ns
BMAd CM vs. BMS + Dex	ns
BMAd CM vs. SBFI + Dex	ns
BMAd CM vs. BMS + SBFI	*
BMAd CM vs. BMS + SBFI + Dex	**
Dex vs. BMS + Dex	ns
Dex vs. SBFI + Dex	ns
Dex vs. BMS + SBFI + Dex	**
BMS vs. BMS + Dex	ns
BMS vs. BMS + SBFI	ns
BMS vs. BMS + SBFI + Dex	*
SBFI vs. SBFI + Dex	ns
SBFI vs. BMS + SBFI	ns
SBFI vs. BMS + SBFI + Dex	*
BMS + Dex vs. BMS + SBFI + Dex	ns
SBFI + Dex vs. BMS + SBFI + Dex	ns
BMS + SBFI vs. BMS + SBFI + Dex	ns

Supplemental Table 2. Combinatorial treatment of dexamethasone, BMS and SBFI induce apoptosis and reduce cell number after 72 hour treatment in MM1S cells. A-D) Statistical importance of treatment with 50 μM BMS, 50 μM SBFI, 80 μM dex and the combinations after 72 hours in MM1S cells.



Supplemental Figure 4. SBFI, dexamethasone, or the combination treated mice maintain weight. A) No weight difference between treatment groups over the duration of study. B) SBFI treated mice have reduced tumor burden compared to vehicle treated mice.