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Ph.D. DISSERTATION DEFENSE

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Date: Wednesday, May 7th, 2025
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Title: Optimizing Multiple Myeloma Treatment Approaches by Elucidating Tumor-Stroma Interactions in the Bone Marrow Microenvironment
Chairperson: Dr. Woo Lee, Department of Chemistry and Chemical Biology, School of Engineering and Sciences

Committee Members: Dr. Adeniyi Lawal, Department of Chemical Engineering and Materials Science, School of Engineering and Sciences
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ABSTRACT

Multiple Myeloma (MM) is a plasma cell malignancy arising in the bone marrow. Despite advancements in therapies, this disease becomes refractory such that relapse and death are inevitable. Immune evasion and drug resistance are partly due to MM-osteoblast cell interactions in the bone marrow microenvironment (BME). We conducted in-vitro studies that provided insights potentially leading to a treatment-relevant hypothesis. Several human MM cell lines (MM.1S, U266, NCI-H929) representing the spectrum of genetic lesions seen in this type of cancer, were co-cultured with osteoblasts for two days to assess proliferation in response to two proteasome inhibitors, bortezomib, and carfilzomib. Osteoblasts, represented by the hFOB fetal osteoblast-derived cell line, markedly enhanced MM cell growth and conferred significant chemoresistance determined by a luciferase-based viability assay. RNA sequencing of FACS-sorted MM and hFOB cells after one day of co-culture revealed a significant upregulation of Type I interferon (IFN-I) signaling target genes (e.g., IRF7, ISG15; $p<0.001$) in MM cells compared to monocultures. We hypothesize that low-level IFN-I signaling, driven by MM–osteoblast interactions via activation of the cGAS-STING pathway, supports MM cell proliferation and survival. In contrast, strong activation of the IFN pathway, either by treatment with recombinant IFN-I or with hypomethylating agents such as decitabine (DAC), induces MM cell death. This biphasic response is consistent with findings observed in other reports. Combination treatment with DAC and recombinant IFN-α demonstrated a synergistic cytotoxic effect on MM cells, suggesting that hyperactivation of IFN signaling promotes MM cell death. Western blot analysis confirmed upregulation of IRF7 protein in MM–osteoblast co-cultures and DAC-treated MM cells, supporting this mechanism. Additionally, treatment with RU.521, a STING pathway inhibitor known to block IFN pathway, disrupted the protective effect provided by osteoblasts in co-culture, further supporting the involvement of low to intermediate level IFN signaling in mediating MM cell survival. Importantly, RNA-seq comparing MM.1S to U266 revealed that, in contrast to MM.1S, U266 cells failed to activate IFN signaling, giving us an entry point for understanding how *TP53* mutations, which are associated with high-risk MM cases, render MM cells independent of the TME. Lastly, two pro-inflammatory cytokines,

CXCL1 and CXCL8, were markedly increased in hFOB co-cultured cells compared to hFOB monocultures. A 9-fold increase of CXCL1 ($p<0.0001$) and CXCL8 ($p<0.0001$) was validated by qPCR, and both chemokines were elevated in co-culture supernatants in a cytokine array and ELISA. These chemokines act on myeloid cells in the TME, which opens another area for future investigations.