MANGANESE PORPHYRIN, MNTE-2-PYP^{5+}, ENHANCES CHEMOTHERAPEUTIC RESPONSE IN HEMATOLOGIC MALIGNANCIES

By

Melba C. Jaramillo

A Thesis Submitted to the Faculty of the

DEPARTMENT OF CELLULAR AND MOLECULAR MEDICINE

In Partial Fulfillment of the Requirements
For the Degree of

MASTERS OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2017

STATEMENT BY AUTHOR

The thesis titled *Manganese Porphyrin, MnTE-2-PyP^{5+}, enhances chemotherapeutic response in hematologic malignancies* prepared by Melba C. Jaramillo has been submitted in partial fulfillment of requirements for a master's degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgement the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Melba C. Jaramillo

APPROVAL BY THESIS DIRECTORS

This thesis has been approved on the date shown below:

_____ 6/26/2017

Margaret E. Tome Date

Assistant Professor, Research Track, Pharmacology

_____ 6/26/2017

Paul St. John Date

Associate Professor, Cellular and Molecular Medicine

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABSTRACT

The prognosis for multiple myeloma (MM) and the activated B-cell subtype of diffuse large B-cell lymphoma (ABC DLBCL) is poor. Gene expression profiling studies have identified that the transcription factor, nuclear factor kappa B (NF-κB) is overexpressed and confers a poor prognosis in MM and ABC DLBCL. NF-κB regulates the transcription of genes involved in cell proliferation and survival. Thus, several groups have tried to identify and/or develop agents that target NF-κB to improve therapy and patient prognosis for MM and ABC DLBCL. Our laboratory has shown that the manganese porphyrin MnTE-2-PyP^{5+} inhibits NF-κB in a murine lymphoma cell culture model and enhances tumor cell death in combination with dexamethasone and cyclophosphamide, two agents that are routinely used to treat these neoplasms. MnTE-2-PyP^{5+} inhibits NF-κB by glutathionylating p65, a member of the NF-κB family. The objective of the following studies was to determine whether MnTE-2-PyP^{5+} enhances the chemotherapeutic response in human MM and ABC DLBCL cells that overexpress and depend on NF-κB for survival. The following studies demonstrate that MnTE-2-PyP^{5+} glutathionylates and inhibits NF-κB in human MM and ABC DLBCL cells. MnTE-2-PyP^{5+} also synergizes with several MM and DLBCL chemotherapeutics, including dexamethasone, cyclophosphamide, vincristine and bortezomib to enhance cell death. The data from these human cell lines will provide the basis for future studies to test MnTE-2-PyP^{5+} in animal models and for translating MnTE-2-PyP^{5+} to the clinic.

CHAPTER ONE: INTRODUCTION

Introduction to hematologic malignancies

Hematologic malignancies are a diverse group of neoplasms that arise from the malignant transformation of bone marrow or lymphatic cells. They are divided into three major groups, including: leukemia, lymphoma and multiple myeloma [1]. According to the American Cancer Society (ACS), more than 137,260 people will be diagnosed with a hematologic malignancy in the United States (U.S) in 2017. Collectively, leukemia, lymphoma and multiple myeloma are predicted to cause the death of more than 58,000 people in the U.S, accounting for nearly 10% of the expected deaths from cancer in 2017.

Leukemia is a cancer of the blood or bone marrow. The ACS estimates that approximately 43,050 new cases of leukemia will be diagnosed in the U.S in 2017. Leukemia is classified on the basis of the cell type involved (lymphoid vs. myeloid) and the state of maturity of the leukemic cell (acute (immature) vs. chronic (mature)) [1]. Acute leukemia is characterized by the rapid proliferation of blasts or immature cells and a rapidly fatal course if untreated. Chronic leukemia is characterized by a proliferation of mature, well-differentiated leukocytes and has a slower clinical course.

Lymphoma is the most common type of hematologic malignancy in the U.S [1]. Lymphomas originate from lymphocytes, including B and T cells. There are two major classes of lymphoma, known as Hodgkin and non-Hodgkin lymphoma (NHL). Hodgkin lymphoma is characterized by the presence of Reed Sternberg cells, which are mature B cells that have become malignant. NHL, by contrast, can be derived from B or T cells and can arise in the lymph nodes as well as in other organs. NHL is more common in the U.S, with more than 72,000

new cases diagnosed each year, compared to 8,000 for Hodgkin lymphoma. NHL is the sixth most common cause of cancer and accounts for approximately 4% of all cancers in the United States. NHL is a heterogeneous group of more than thirty lymphoid neoplasms, varying from indolent to aggressive disease [2]. The most frequently diagnosed NHLs are diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma [3]. DLBCL is an aggressive lymphoma of B cells that accounts for approximately forty percent of lymphomas; whereas, follicular lymphoma is a slow-growing, indolent B-cell lymphoma [3]. Follicular lymphoma accounts for approximately twenty percent of the NHLs diagnosed each year [3].

Multiple myeloma (MM) is caused by the proliferation and accumulation of malignant plasma cells [1]. MM causes extensive skeletal destruction resulting in anemia, bone pain, and fractures. It accounts for 2% of all cancers in the U.S and is the second most common hematologic malignancy in the U.S. The ACS predicts that in 2017 there will be over 14,400 new cases of MM in the U.S.

Hematologic malignancies are commonly treated with chemotherapy. There are currently more than fifty different chemotherapeutic agents used to treat patients with hematologic malignancies [4]. The standard treatment for DLBCL and follicular lymphoma includes cyclophosphamide (C), doxorubicin (H), vincristine (O), prednisone (P; a synthetic glucocorticoid), and the monoclonal antibody Rituximab (R) [5, 6], which are collectively referred to as R-CHOP. The standard treatment for MM consists of cyclophosphamide, dexamethasone (another synthetic glucocorticoid), and the proteasome inhibitor, bortezomib.

Unfortunately, one of the biggest challenges facing clinicians is the heterogeneous response to therapy. While existing therapies improve patient survival for some types of hematologic malignancies, not all respond well to treatment. In DLBCL, for example, 50% of

patients respond, 25% initially respond then relapse, while 25% show no response to R-CHOP [5, 6]. The response to R-CHOP in follicular lymphoma is also poor; less than 40% of patients respond to treatment and of the 40% that do respond, over 60% eventually develop resistance [7]. In MM, most patients also become refractory to treatment and eventually die. The five-year survival rate for MM is less than 5% [8]. To improve patient outcome, therefore, it is important to identify potential targets to improve treatment and to ultimately develop novel agents to improve patient survival.

Modulation of the redox environment as a therapeutic target for hematologic malignancies

The redox environment has emerged as a promising target for anti-cancer drug discovery [9]. Mounting evidence suggests that, compared with their normal counterparts, many types of cancer cells have increased levels of reactive oxygen species (ROS) [10-12]. For example, leukemia cells isolated from blood samples from patients showed increased ROS production compared with normal lymphocytes [9, 10]. In normal cells, a moderate increase in ROS is associated with increased proliferation [13]. Normal cells have the appropriate checks and balances to control and regulate the effects of increased proliferation due to increased ROS. An increase in ROS in tumor cells, on the other hand, induces cell death since the tumor cells are closer to the apoptotic threshold [13]. The differential ROS between normal and cancer cells represents a specific vulnerability in cancer cells and provides a therapeutic window that can be targeted by redox modulating drugs. Consequently, several groups have proposed that cancer cells with increased oxidative stress are likely to be more vulnerable to damage by further ROS insults [9, 10].

Several standard chemotherapeutics including anthracyclins, bleomycin, bortezomib, and glucocorticoids have been shown to increase intracellular ROS [9, 14, 15]. The increased ROS is

may contribute to their chemotherapeutic efficacy. For example, in lymphoid malignancies, glucocorticoid treatment contributes to the therapeutic effect [14]. Our laboratory has shown, glucocorticoids increase the levels of H_2O_2 [14]. The amplitude of the H_2O_2 signal determines the sensitivity of the cells to glucocorticoids. On the other hand, the ROS produced by anthracyclins may not contribute to the cell killing of lymphoma cells [16-18]. The amount or species of ROS produced may be insufficient to contribute to the therapeutic effect. These data suggest that manipulation of the redox environment may be a promising target to improve treatments for hematologic malignancies.

Manganese porphyrins act as pro-oxidants

The understanding that cancer cells can be targeted by ROS-mediated mechanisms has been accompanied by an increased search for synthetic compounds that can modulate the cellular redox environment. One class of redox-modulating compounds being extensively studied is the manganese porphyrins. Manganese porphyrins have been shown to act as redox cycling agents in cell-free systems [19]. The manganese at the center of the porphyrin ring is redox sensitive. In the test tube, the manganese can achieve four oxidation states: Mn(II), Mn(III), Mn(IV), and Mn(V) [19-21]. In cell-free systems, manganese porphyrins are most stable in the Mn(III) oxidation state. In this state, the porphyrin donates electrons, acts as a reducing agent, and scavenges radicals. When the porphyrin donates electrons, the redox-potential of the manganese becomes highly positive and it increases the porphyrins' affinity for electrons [19]. Thus, manganese porphyrins are easily oxidized to Mn(IV). In this state, manganese porphyrins lose their reducing abilities and instead accept electrons and act as oxidizing agents.

The above studies have also shown that the manganese, although surrounded by porphyrin rings, is exposed and accessible to other cellular components [22]. In cell-free

systems, manganese porphyrins have been shown to interact and redox cycle with flavin oxidoreductases, such as complex I in the mitochondria and the cytochrome P450 complex [23, 24]. They will also interact with and deplete the reducing power of small molecule reductants such as ascorbate, tetrahydrobiopterin, and glutathione (GSH) [22, 23, 25]. Manganese porphyrins depend on these small molecule reductants to reduce the active site manganese from the Mn(IV) to the Mn(III) state.

The ability of manganese porphyrins to act as redox-cycling agents in a test tube suggests that they may also be able to act as such in cell and animal models. *In vitro*, manganese porphyrins can modulate ROS-based signaling pathways, such as AP-1, HIF1α and NF-κB [26-29]. In skin and lung carcinoma animal models, manganese porphyrins inhibit the transcription factors AP-1 and HIF1α [27]. Manganese porphyrins have also been shown to inhibit DNA binding of NF-κB in an animal model of immune response [28]. These pathways are important for cell survival and proliferation and are implicated in cancer.

Pharmacokinetic studies in mouse models have also demonstrated that manganese porphyrins can reach multiple tissues at concentrations high enough to be catalytically active without causing toxic side effects [30]. Data from a clinical trial with a manganese porphyrin in amyotrophic lateral sclerosis patients did not report any side effects [31]. The efficacy and safety of manganese porphyrins, as well as their ability to modulate redox-sensitive signaling processes *in vitro* and *in vivo* suggests that they may be able to manipulate the redox environment and act as adjuvants in hematologic malignancies.

Therapeutic potential of manganese porphyrins in hematologic malignancies

Our work in a lymphoma cell culture model confirms that the manganese porphyrin, manganese (III) *meso*-tetrakis (N-ethylpyridinium-2-yl) porphyrin ($MnTE\text{-}2\text{-}PyP^{5+}$), acts as a

pro-oxidant. In WEHI7.2 murine thymic lymphoma cells MnTE-2-PyP^{5+} acts as a pro-oxidant in the presence of increased H_2O_2 levels or in an oxidized redox environment [32]. MnTE-2-PyP^{5+} acts as a pro-oxidant in these cells by depleting GSH, increasing glutathione disulfide, and glutathionylating redox sensitive proteins [32]. Furthermore, we found that MnTE-2-PyP^{5+} glutathionylates the redox sensitive transcription factor nuclear factor kappa B (NF-κB) and inhibits NF-κB activity in WEHI7.2 cells [32]. We also found that MnTE-2-PyP^{5+} enhances cyclophosphamide and glucocorticoid-induced apoptosis in murine lymphoma cells and has no effect on doxorubicin or vincristine toxicities [33]. In H9c2 mouse cardiomyocytes, MnTE-2-PyP^{5+} protected heart cells from doxorubicin toxicity, which is one of the major dose limiting factors for doxorubicin use in the clinic [33]. Furthermore, in peripheral blood mononuclear cells (PBMC), the normal counterpart of lymphoma cells, MnTE-2-PyP^{5+} does not enhance glucocorticoid-induced apoptosis, suggesting that the ability of MnTE-2-PyP^{5+} to enhance cell death is specific to tumor cells [34]. Collectively, our data in the WEHI7.2 cells suggest that MnTE-2-PyP^{5+} has potential as a novel lymphoma chemotherapeutic, especially in cells that are highly oxidized and/or depend on NF-κB for survival.

Targeting NF-κB in hematologic malignancies

NF-κB is a redox sensitive transcription factor that regulates survival pathways in lymphoid cells. Activation of the canonical NF-κB pathway results in the formation of p65/p50 heterodimers that translocate to the nucleus, bind DNA and activate transcription. All members of the NF-κB family contain a DNA binding domain within the conserved Rel homology domain [35]. Several members contain an extra 300 amino acid sequence at the C-terminus that is important for transactivation [35]. The p65 NF-κB subunit, for example, contains both a DNA binding domain and C-terminus amino acid sequence that is important for transactivation and

gene transcription [35]. Downstream targets of NF-κB include increases in anti-apoptotic proteins, such as Bcl-2, Bcl-X_L, and P-glycoprotein, a transport protein involved in drug efflux [35]. Increases in anti-apoptotic proteins and P-glycoprotein are implicated in chemotherapy resistance.

Gene expression profiling studies in DLBCL cells and tissue have shown that there are two major subtypes of DLBCL: activated B-cell like (ABC) and germinal center B-cell like (GCB) DLBCL [2, 36]. ABC DLBCL exhibits a dependence on constitutively active NF-κB and has a worse overall survival compared to GCB DLBCL [36]. GCB DLBCL is characterized by an increased number of mutations in histone modifying genes and a better overall survival [36]. Multiple myeloma cells and tissue have also been shown to overexpress and to depend on NF-κB for survival [37]. These studies suggest that use of agents that target NF-κB and that can be used in combination with the existing therapeutics for DLBCL and MM have the potential to improve patient outcome.

Statement of the Problem

The prognoses for MM and ABC DLBCL are poor. Gene expression profiling studies have revealed that NF-κB, a critical survival protein, is constitutively active in MM and ABC DLBCL, and contributes to the poor prognosis [36, 37]. Use of agents that target NF-κB have the potential to improve patient outcome. Our laboratory previously demonstrated that MnTE-2-PyP^{5+}, a redox cycling agent, acts as a pro-oxidant to enhance glucocorticoid-induced apoptosis in WEHI7.2 murine lymphoma cells [32]. An increase in H_2O_2 and the presence of GSH are critical for the porphyrin's ability to cause this effect [32]. Alone, MnTE-2-PyP^{5+} was neither able to increase oxidative stress nor induce apoptosis. In an oxidized redox environment, however, MnTE-2-PyP^{5+} redox cycles with GSH, glutathionylates the p65 NF-κB subunit, and

inhibits NF-κB activity in murine lymphoma cells [32]. These studies suggest that MnTE-2-PyP^{5+} enhances apoptosis in oxidized environments or in combination with other agents that increase H_2O_2 levels, and in cells that overexpress NF-κB.

To generate preclinical data for translating MnTE-2-PyP^{5+} to the clinic, it is important to measure the effects of MnTE-2-PyP^{5+} on human DLBCL and MM cells that depend on NF-κB. The objective of the following study was to determine to what extent our observations in the murine lymphoma cells translate to human DLBCL and MM cells. Data from human cell lines will provide the basis for future studies to test MnTE-2-PyP^{5+} in animal models. The hypothesis tested in this project is that MnTE-2-PyP^{5+} will enhance the chemotherapeutic response in hematologic malignancies that overexpress and depend on NF-κB for survival.

CHAPTER TWO: MATERIALS AND METHODS

Cell Culture- The following human DLBCL cells were obtained from Dr. Lisa Rimsza (University of Arizona, Tucson, AZ): SUDHL4, OCILY19, U2932, and OCILY3 cells. The SUDHL4 and OCILY19 cells are GCB DLBCL cells and the U2932 and OCILY3 are ABC DLBCL cells. 8226 and MM.1S multiple myeloma cells were obtained from Dr. Terry Landowski (University of Arizona). A20 murine B cell lymphoma cells were obtained from Dr. Yi Zeng (University of Arizona); they have an inactivating A20 mutation that results in constitutively active NF-κB [38]. All cells were maintained in suspension in RPMI 1640 Medium (Mediatech Inc., Manassas, VA) supplemented with 10% fetal bovine serum (ATCC, Manassas, VA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA) at 37ºC in a 5% CO_2 humidified environment.

Drugs and Drug Treatments- The manganese porphyrin, manganese (III) *meso*-tetrakis (N-ethylpyridinium-2-yl) porphyrin (MnTE-2-PyP^{5+}) was provided by Dr. Ines Batinic-Haberle (Duke University, Durcham, NC). In the following studies, we treated the cells with 50 nM MnTE-2-PyP^{5+} because previously we demonstrated that in WEHI7.2 cells 50 nM MnTE-2-PyP^{5+} does not induce cell death on its own but enhances the effect of several drugs used to treat lymphoma and MM. All other drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

The concentrations of cyclophosphamide, doxorubicin, vincristine, dexamethasone and bortezomib used in DLBCL cells for this study were selected based on the EC_{50} values for each cell line. The EC_{50} for cyclophosphamide in the SUDHL4 cells was 3.85 ± 0.21 nM; 4.07 ± 0.11 nM in the OCILY19; and 4.88 ± 0.19 nM in the U2932 cells. The EC_{50} for doxorubicin in the

SUDHL4 cells was 14.56 ± 0.32 nM; 14.97 ± 0.14 nM in the OCILY19; and 17.02 ± 0.26 nM in the U2932 cells. The EC_{50} for vincristine in the SUDHL4 cells was 5.10 ± 0.10 μM; 7.34 ± 0.13 μM in the OCILY19; and 6.22 ± 0.08 μM in the U2932 cells. The EC_{50} for dexamethasone in the SUDHL4 cells was 25.41 ± 0.41 μM; 30.06 ± 0.34 μM in the OCILY19; 51.02 ± 0.29 μM in the U2932 cells; 45.11 ± 0.67 μM in the OCILY3 cells; and 5.03 ± 0.44 nM in the A20 cells. These concentrations were used to test the effect of MnTE-2-PyP^{5+} in combination with the CHOP reagents in DLBCL cells. The concentration of bortezomib tested in DLBCL cells was 3.12 ± 0.19 nM in the SUDHL4; 3.05 ± 0.32 nM in the OCILY19; and 3.45 ± 0.33 nM in the U2932 cells. Furthermore, the EC_{50} for dexamethasone in the A20 B cell lymphoma cells was 5.15 ± 0.08 nM. These concentrations were used to test the effect of MnTE-2-PyP^{5+} in combination with these agents.

The same approach was used to determine the sensitivity of MM cells to dexamethasone and bortezomib. The EC_{50} for dexamethasone in the 8226 and MM.1S cells was 12.51 ± 0.75 μM and 15.01 ± 0.45 μM, respectively. The EC_{50} for bortezomib was 5.25 ± 0.10 nM and 4.97 ± 0.08 nM in the 8226 and MM.1S cells, respectively.

Protein measurements- Total cellular protein was measured in clarified lysates using the BCA Protein Assay Kit (Pierce, Rockford, IL) according to the manufacturer's instructions, as previously described [32].

Cell Viability Measurements- To determine whether MnTE-2-PyP^{5+} enhances cell death in combination with the CHOP reagents and bortezomib in DLBCL, MM, and A20 cells, the relative cell number was measured after 48 hours of treatment with or without 50 nM MnTE-2-PyP^{5+} using the Non-Radioactive Cell Proliferation (MTS) Assay (Promega Corp., Madison, WI). Plates were read at 490 nm using a Synergy HT plate reader (BioTek Instruments,

Winooski, VT). A decrease in the EC_{50} for the drug in the presence of MnTE-2-PyP^{5+} compared to that in the absence of MnTE-2-PyP^{5+} was interpreted as increasing sensitivity.

Cell Death Measurements- To determine the ability of MnTE-2-PyP^{5+}, dexamethasone, bortezomib, or the combination treatments to induce apoptosis in DLBCL and MM cells, cells were treated with 50 nM MnTE-2-PyP^{5+}, and/or the EC_{50} of dexamethasone or bortezomib for 24 hours and stained with 7-AAD or propidium iodide (PI) (R&D Systems, Inc., Minneapolis, MN). Cellular fluorescence for 7-AAD or PI was measured and analyzed using an EPIC-XL-MCL flow cytometer (Coulter Corp., Miami, FL). Cells that were positive for 7-AAD or PI were considered dead. Ten thousand cells were analyzed per sample. Numbers less than 5% different were considered within the error of the machine after calibration for this assay.

Amplex Red™ Measurements- H_2O_2 levels were measured using the horseradish peroxidase (HRP)-linked fluorometric indicator Amplex Red™ (Invitrogen, Carlsbad, CA) as described previously in Jaramillo et al. [32]. Briefly, cells were resuspended in phenol-red free RPMI 1640 medium containing 50 μM Amplex Red™ and 0.1 unit/mL horseradish peroxidase. The rate at which Amplex Red™ fluorescence increased (Ex: 485/Em: 510) over a 4-hour period was measured using a Synergy HT plate reader (Bio Tek Instruments, Inc.). Values were normalized for cellular protein.

Immunoblots- To measure the overall protein glutathionylation, 25 μg protein from clarified total cell lysates was separated by molecular weight using SDS-PAGE. Blots were probed with an anti-GSH antibody (Virogen, Watertown, MA) at a 1:2,500 dilution followed by a 1:2,500 dilution of horseradish peroxidase-linked goat anti-mouse Ig (GE Healthcare, Pacataway, NJ). Blots were also probed with an anti-ß actin antibody (AbCam, Cambridge, MA) at a 1:50,000 dilution followed by a 1:2,500 dilution of horseradish peroxidase-linked goat anti

mouse Ig (AbCam) as a loading control. To visualize multiple bands on the same blot, Restore Western Blot Stripping Buffer (Pierce; ThermoFischer, Waltman, MA) was used before being probed with a new antibody. Proteins were visualized by chemiluminescence.

NF-κB Glutathionylation- Co-immunoprecipitations were done using the Pierce Co-Immunoprecipitation Kit (Pierce; ThermoFischer, Waltman, MA) using the manufacturer's protocol. Briefly, 10 μg of the p65 NF-κB antibody (AbCam) was linked to 25 μL resin. Samples were lysed in the kit lysis buffer and protein concentration measured using the BCA Protein Assay kit. The sample was pre-cleared using the control agarose resin and the sample incubated with the antibody-linked or control resin overnight at 4°C. Samples were eluted and prepared for gel electrophoresis according to the manufacturer's instructions. Proteins were separated by SDS-PAGE for immunoblot analysis. Blots were probed with an anti-GSH antibody (Virogen) to determine whether they are glutathionylated, or with a p65 antibody (AbCam) to control for loading.

Quantitation of Immunoblot Proteins and Statistics- Means were compared using student's t-tests with the algorithm in Excel (Microsoft Corp., Redmond, WA). Means were considered significantly different when $p \leq 0.05$.

CHAPTER THREE: RESULTS

MnTE-2-PyP^{5+} alone increases cell death in ABC DLBCL cells. To generate preclinical data necessary for translating MnTE-2-PyP^{5+} to the clinic, we measured the effect of MnTE-2-PyP^{5+} on human DLBCL cells. ABC DLBCL cells have been shown to overexpress and depend on NF-κB for survival [36]; therefore, we hypothesized that treatment with MnTE-2-PyP^{5+} alone would cause some cell death in ABC DLBCL cells, while GCB cells that do not depend on NF-κB would not have a similar response. We measured the number of viable cells following treatment with 50 nM MnTE-2-PyP^{5+} for 48 hours in ABC and GCB DLBCL cells using the MTS assay, which measures the number of metabolizing cells, and by staining the cells with propidium iodide (PI). Cells that stained positive for PI were considered dead. We found that MnTE-2-PyP^{5+} alone decreased cell viability by approximately 25% and 35% in the U2932 and OCILY3 cells, respectively (Figure 1). The GCB cells (SUDHL4 and OCILY19), on the other hand, did not show an increase in cell death due to MnTE-2-PyP^{5+} treatment (Figure 1).

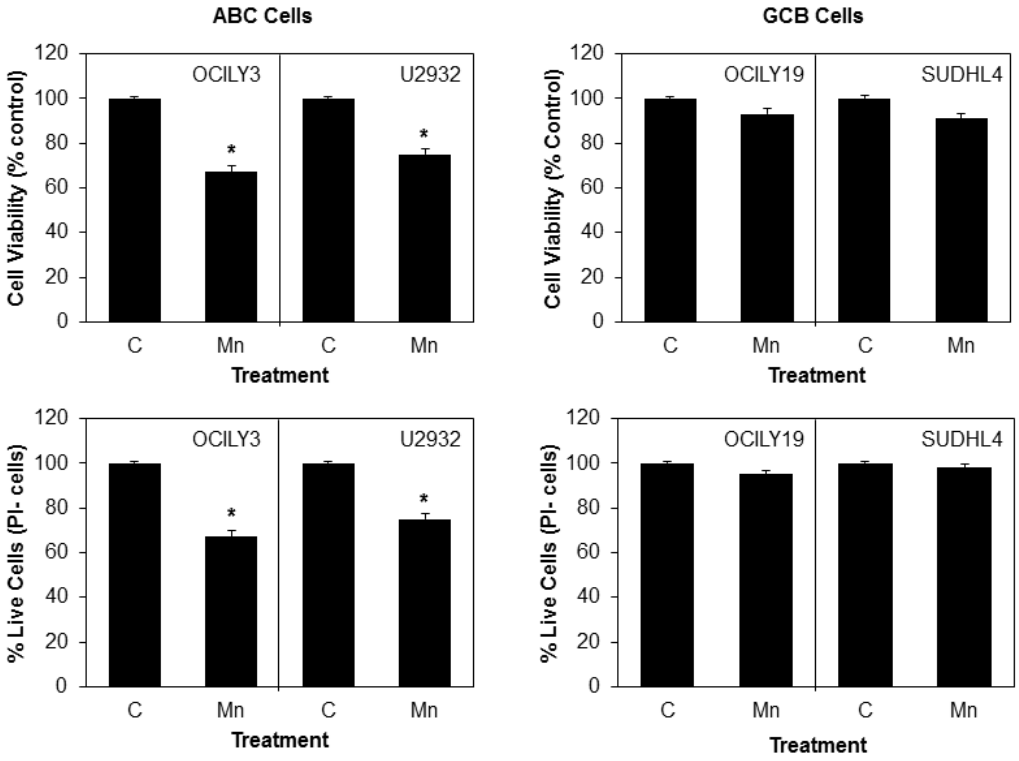

Figure 1. MnTE-2-PyP^{5+} increases cell death in ABC DLBCL cells. ABC (OCILY3 and U2932) and GCB (OCILY19 and SUDHL4) cells were treated with 50 nM MnTE-2-PyP^{5+} (Mn) or treated with a vehicle control (C) for 48 hours. Cell viability was measured by the MTS assay and the % live cells by flow cytometry. * indicates significantly different from control treated cells, $p \leq 0.05$. Values are the mean + S.E.M (n=6)

MnTE-2-PyP^{5+} enhances cell death in the presence of H_2O_2. Next, we tested whether MnTE-2-PyP^{5+} enhanced glucocorticoid-induced cell death in ABC and GCB DLBCL cells. In combination with dexamethasone, a synthetic glucocorticoid, MnTE-2-PyP^{5+} enhanced dexamethasone-induced cell death in both GCB and ABC DLBCL cells (Figure 2). Given the importance of H_2O_2 for the porphyrin's ability to enhance dexamethasone-induced cell death in WEHI7.2 cells, we measured the rate of H_2O_2 efflux due to dexamethasone treatment in the DLBCL cells (Figure 2). H_2O_2 readily diffuses through the plasma membrane so the rate of efflux is proportional to the intracellular H_2O_2 concentration. Dexamethasone treatment for 24 hours enhanced the H_2O_2 efflux in DLBCL cells. Compared to control cells, MnTE-2-PyP^{5+} alone did not augment H_2O_2 levels and there was not a difference in H_2O_2 efflux between cells treated with dexamethasone alone and cells treated with the combination of MnTE-2-PyP^{5+} plus dexamethasone (data not shown).

We also tested whether bortezomib, an experimental drug in DLBCL, increased H_2O_2 in these cells and whether MnTE-2-PyP^{5+} enhanced death due to bortezomib. The efficacy of bortezomib is reportedly partially dependent on an oxidative mechanism [37]. As proof of principle, we found that treatment with bortezomib also increased H_2O_2 and that MnTE-2-PyP^{5+} increased bortezomib-induced cell death in ABC DLBCL cells. Treatment with bortezomib for 24 hours increased H_2O_2 efflux 2.7 ± 0.1-fold in U2932 cells and killed 51.26 ± 0.33% cells. In combination with bortezomib, MnTE-2-PyP^{5+} increased the number of dead U2932 cells to 78.92 ± 0.14%. Taken together these data suggest that MnTE-2-PyP^{5+} enhances DLBCL cell death in combination with drugs that increase H_2O_2.

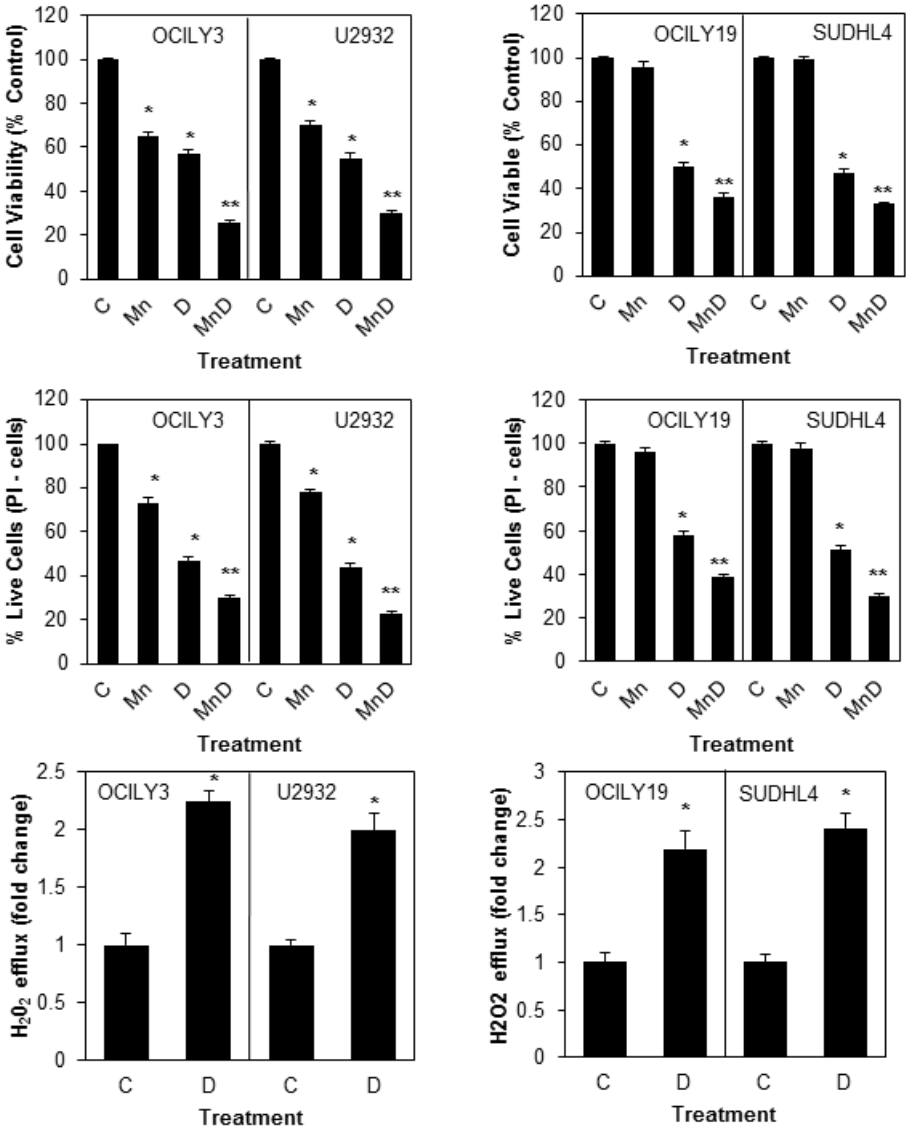

Figure 2. MnTE-2-PyP^{5+} enhances apoptosis in the presence of H_2O_2. ABC (OCILY3 and U2932) and GCB (OCILY19 and SUDHL4) cells were treated with a vehicle control (C), 50 nM MnTE-2-PyP^{5+} (Mn), Dexamethasone (D) or Mn and D together (MnD) for 48 hours. Cell viability was measured by the MTS assay and the % live cells by flow cytometry. H_2O_2 efflux was measured using Amplex Red. * indicates significantly different from control treated cells and ** indicates significantly different from dexamethasone-treated cells, $p \leq 0.05$. Values are the mean + S.E.M. (n=3).

MnTE-2-PyP^{5+} enhances glucocorticoid-induced cell death in A20 B-cell lymphoma cells. In ABC DLBCL, NF-κB dependence is caused by several mechanisms including A20 inactivating mutations [38]. A20 (also known as TNFAIP3) functions as a ubiquitin-editing enzyme and is required for termination of NF-κB responses in the classical NF-κB pathway [38]. Several reports suggest that more than 30% of ABC DLBCLs have inactivating A20 mutations [39]. The murine A20 cells have an inactivating mutation in A20, and thus have constitutively active NF-κB. We used this cell line to test whether the porphyrin alone or in combination with dexamethasone could induce cell death on its own and increase dexamethasone-induced cell death. Treatment with 50 nM MnTE-2-PyP^{5+} for 48 hours decreased the percentage of viable cells by approximately 28% and in combination with 5 nM dexamethasone, MnTE-2-PyP^{5+} enhanced dexamethasone-induced cell death by 28% (Figure 3).

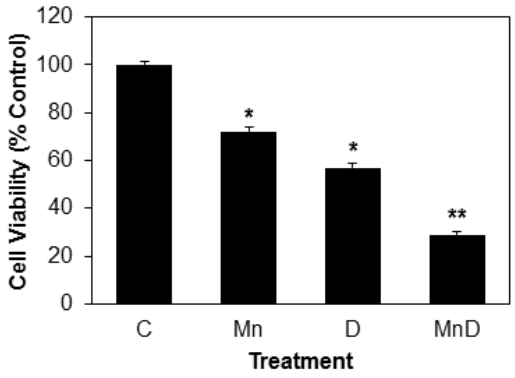

Figure 3. MnTE-2-PyP^{5+} enhances glucocorticoid-induced apoptosis in A20 B-cell lymphoma cells. A20 lymphoma cells were treated with a vehicle control (C), 50 nM MnTE-2-PyP^{5+} (Mn), 5 nM dexamethasone (D), or Mn in combination with D (MnD) for 48 hours. Cell viability was measured using the MTS assay. * indicates significantly different from control treatment and ** indicates significantly different from dexamethasone treatment, $p \leq 0.05$. Values are the mean + S.E.M. (n=3)

MnTE-2-PyP^{5+} increases NF-κB glutathionylation in the presence of dexamethasone in DLBCL. Based on our previous data from the WEHI7.2 cells [32], we tested whether the porphyrin enhances protein glutathionylation. We found that in U2932 and A20 cells, MnTE-2-PyP^{5+} enhanced total protein glutathionylation (Figure 4A), which is a reversible post-translational modification of redox sensitive cysteines on proteins. Glutathionylation occurs when the amount of oxidized glutathione increases and/or when the redox environment of the cell is oxidized [40]. Protein glutathionylation is a physiologically relevant mechanism for controlling the activity of redox-sensitive proteins and is usually inhibitory [40].

NF-κB is a redox-sensitive transcription factor that regulates the expression of wide variety of anti-apoptotic genes. The activity of NF-κB depends on the oxidation state of its cysteine residues [40]. The cysteines must be in a reduced state for NF-κB to bind DNA and to activate gene transcription [40]. These residues are surrounded by a cationic environment that makes the cysteines very reactive and susceptible to oxidation or glutathionylation [40]. In WEHI7.2 cells, we demonstrated that MnTE-2-PyP^{5+} and MnTE-2-PyP^{5+} in combination with dexamethasone, glutathionylates the p65 NF-κB subunit and inhibits NF-κB activity more than SN50, a well-established NF-κB inhibitor [32].

We tested for glutathionylation of the p65 NF-κB subunit in U2932 cells treated for 16 hours with dexamethasone in the absence or presence of MnTE-2-PyP^{5+}. Whole cell lysates were immunoprecipitated for p65 and then immunobloted using an anti-GSH antibody to measure their respective glutathionylation. Figure 4B demonstrates that treatment with MnTE-2-PyP^{5+} and MnTE-2-PyP^{5+} combined with dexamethasone increased glutathionylated p65 in U2932 cells. MnTE-2-PyP^{5+} increased p65 glutathionylation to nearly 2-fold over that in the vehicle-control. Dexamethasone treatment also increased glutathionylation of p65 but only to 1.5-fold

the amount in the vehicle-control. The combination treatment enhanced p65 glutathionylation 2.4-fold the value in the vehicle-treated cells. Pretreating these cells with 60 μM buthionine sulfoximine (BSO), a glutathione synthesis inhibitor, attenuated the ability of the porphyrin in combination with dexamethasone, to glutathionylate p65 (Figure 4A & B).

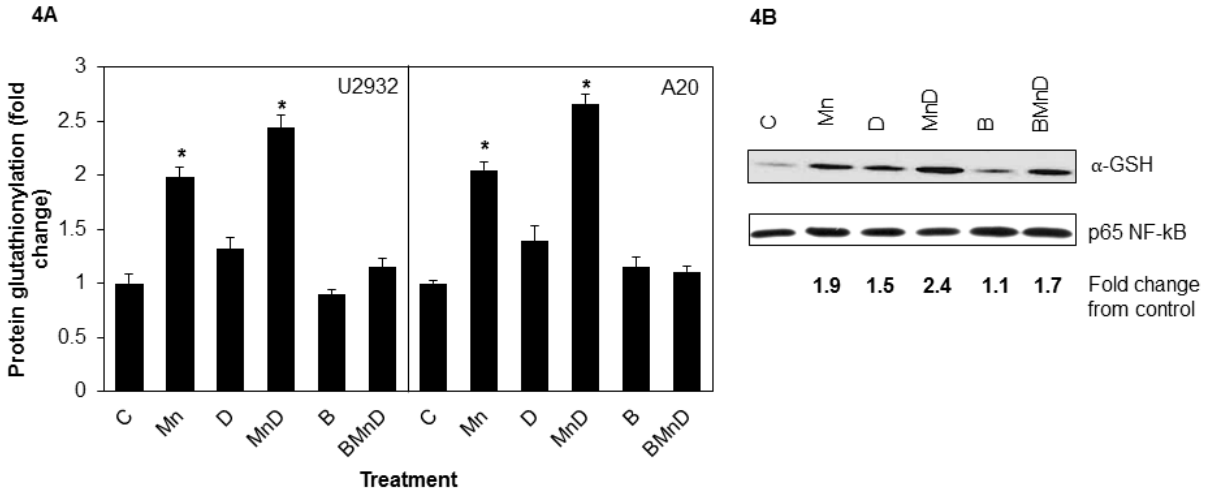

Figure 4. MnTE-2-PyP^{5+} induces protein glutathionylation. ***A. Overall protein glutathionylation.*** U2932 and A20 cells were treated with a vehicle control (C), 50 nM MnTE-2-PyP^{5+} (Mn), Dexamethasone (D), MnTE-2-PyP^{5+} in combination with dexamethasone (MnD), BSO (B; a glutathione synthesis inhibitor) and BSO in combination with MnD (BMnD) for 16 hours. Following treatment, the overall protein glutathionylation was determined by Western blot using an anti-GSH antibody. * indicates significantly different from control or dexamethasone treatment. Values are the mean + S.E.M (n=3); $p \leq 0.05$. ***B. p65 NF-κB glutathionylation.*** The glutathionylation of the p65 NF-κB subunit in U2932 cells was also measured by western blot. Shown here is a representative immunoblot (n=3) of the glutathionylation of the p65 subunit following treatment with a vehicle control (C), 50 nM MnTE-2-PyP^{5+} (Mn), Dexamethasone (D), MnTE-2-PyP^{5+} in combination with dexamethasone (MnD), BSO (B), and BSO in combination with MnD (BMnD) for 16 hours. Fold change is the for the mean of 2 immunoblots.

Effect of MnTE-2-PyP^{5+} on cell death due to other CHOP drugs. To use MnTE-2-PyP^{5+} as an adjuvant it is necessary to test the interaction of MnTE-2-PyP^{5+} with the remaining agents in the CHOP regimen, including cyclophosphamide, doxorubicin, and vincristine. Testing MnTE-2-PyP^{5+} in combination with doxorubicin is particularly crucial because its use in the clinic is often limited by acute and chronic cardiotoxicity [41]. As shown in Table 1, treatment with 50 nM MnTE-2-PyP^{5+} for 48 hours enhanced cell death due to vincristine and cyclophosphamide and did not affect doxorubicin sensitivity in the ABC and GCB DLBCL cells. Collectively, these results suggest that MnTE-2-PyP^{5+} has potential as a novel therapeutic for lymphomas, especially those overexpressing NF-κB.

Cell Type	MnTE-2-PyP^{5+} (nM)	Cyclophos-phamide EC_{50} (nM)	Doxorubicin EC_{50} (nM)	Vincristine EC_{50} (μM)
SUDHL4	0	3.85 ± 0.21	14.56 ± 0.32	5.10 ± 0.10
	50	2.02 ± 0.16*	14.77 ± 1.09	3.48 ± 0.31
OCILY19	0	4.07 ± 0.11	14.97 ± 0.14	7.34 ± 0.13
	50	2.14 ± 0.34*	15.19 ± 1.80	5.79 ± 0.42*
U2932	0	4.88 ± 0.19	17.02 ± 0.26	6.22 ± 0.08
	50	2.11 ± 0.09*	17.19 ± 2.10	4.02 ± 0.07*

Table 1. MnTE-2-PyP^{5+} synergizes with cyclophosphamide and vincristine. GCB (SUDHL4 and OCILY19) and ABC (U2932) cells were treated with cyclophosphamide, doxorubicin, or vincristine alone or in combination with 50 nM MnTE-2-PyP^{5+} for 48 hours. Cell viability was measured using the MTS assay. * indicates significantly different from treatment with cyclophosphamide or vincristine only, $p \leq 0.05$. Values are the mean + S.E.M. (n=3)

MnTE-2-PyP^{5+} enhances dexamethasone and bortezomib-induced cell death in MM cells. MM is another hematological malignancy that overexpresses NF-κB and has a poor clinical prognosis. Two of the standard drugs for MM treatment are dexamethasone and bortezomib. Previously, Brown et al., have shown that dexamethasone increases H_2O_2 levels in MM cells [42]. In this study, we determined whether bortezomib increases H_2O_2 levels in MM cells using Amplex Red^{TM}. Treatment with bortezomib for 24 hours increased H_2O_2 efflux 1.7 ± 0.1-fold in 8226 and 1.6 ± 0.2-fold in MM.1S cells. Furthermore, treatment with 50 nM MnTE-2-PyP^{5+} for 48 hours decreased the percentage of viable MM cells alone and in combination with dexamethasone or bortezomib (Figure 5). These findings suggest that MnTE-2-PyP^{5+} also has potential to improve therapy for MM.

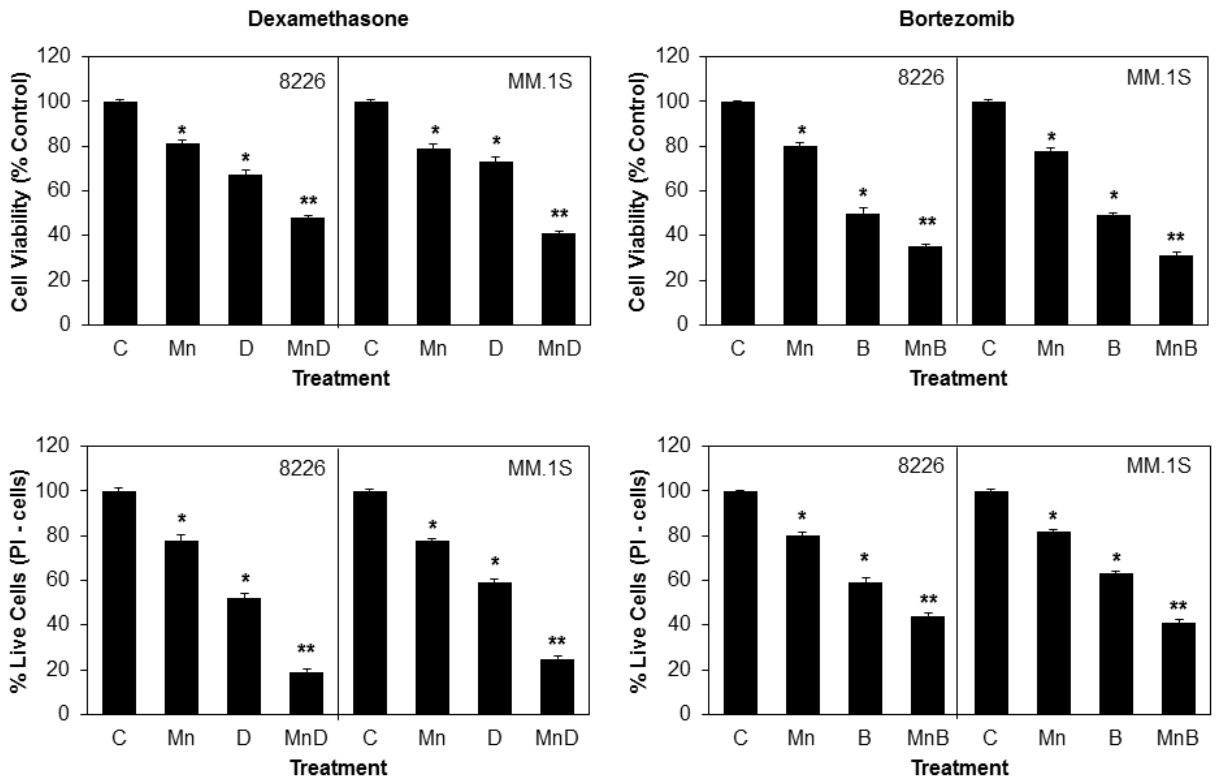

Figure 5. MnTE-2-PyP^{5+} enhances cell death in MM cells in combination with dexamethasone or bortezomib. 8226 and MM.1S cells were treated with a vehicle control (C), 50 nM MnTE-2-PyP^{5+} (Mn), dexamethasone (D) or bortezomib (B), and Mn in combination with D (MnD) or B (MnB) for 48 hours. Cell viability was measured using the MTS assay and the % live cells by flow cytometry. * indicates significantly different from control treatment and ** indicates significantly different from dexamethasone or bortezomib treatment, $p \leq 0.05$. Values are the mean + S.E.M. (n=6)

Dexamethasone treatment does not increase H_2O_2 levels in normal cells. Previously we found that MnTE-2-PyP^{5+} does not increase cell death due to dexamethasone in normal PBMC [33]. If H_2O_2 is critical for the porphyrin's ability to enhance cell death, we hypothesized that in the PBMC, dexamethasone treatment does not increase the levels of H_2O_2. As expected, the levels of H_2O_2 did not increase due to dexamethasone treatment in PBMC (Figure 6).

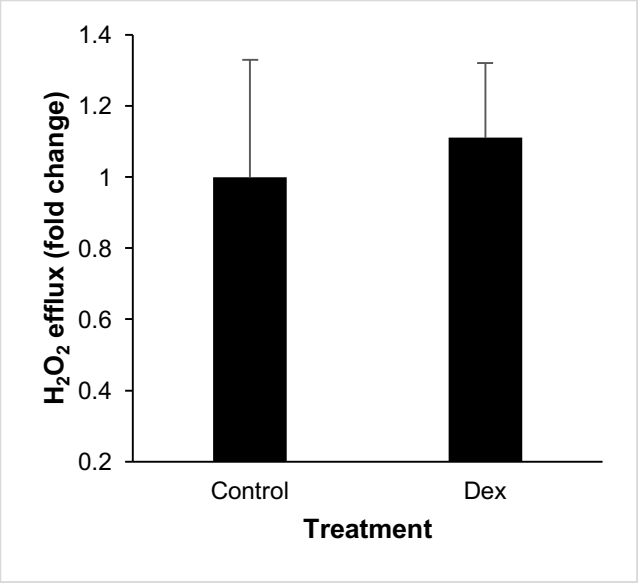

Figure 6. Dexamethasone treatment does not increase H_2O_2 levels in normal cells. Normal human PBMC were treated with a vehicle control or Dexamethasone (Dex) for 24 hours. H_2O_2 efflux was measured using Amplex Red. Values are the mean + S.E.M. (n=3).

CHAPTER FOUR: DISCUSSION AND FUTURE DIRECTIONS

Our data suggest that MnTE-2-PyP^{5+} has potential as a novel therapeutic for hematologic malignancies, especially those that have constitutively active NF-κB. Constitutively active NF-κB activity confers a poor prognosis in hematologic malignancies, including in ABC DLBCL and MM. As a single agent, MnTE-2-PyP^{5+} decreased the viable cell number in murine A20 B-cell lymphoma cells, U2932 human ABC DLBCL and in MM cell lines, which overexpress and depend on NF-κB for survival. The porphyrin also synergized with agents used to treat hematologic malignancies, including dexamethasone, cyclophosphamide, vincristine, and bortezomib. MnTE-2-PyP^{5+} treatment however, did not increase cell death in normal peripheral blood mononuclear cells (PBMC), suggesting that MnTE-2-PyP^{5+} may be selective to tumor cells.

Previously, our laboratory demonstrated that the mechanism by which MnTE-2-PyP^{5+} enhances dexamethasone-induced apoptosis in WEHI7.2 cells involves inhibition of NF-κB through glutathionylation of the p65 NF-κB subunit [32]. Consistent with these findings, in the current study, we demonstrate that cells that overexpress and depend on NF-κB are more sensitive to MnTE-2-PyP^{5+}. Treatment with MnTE-2-PyP^{5+} alone induced cell death in A20, U2932, OCILY3, 8226, and MM.1S cells, which have been shown to overexpress active NF-κB. On the other hand, in cells that do not have constitutively active NF-κB, MnTE-2-PyP^{5+} did not induce cell death as a single agent. Furthermore, we also found that the porphyrin glutathionylates redox sensitive proteins in U2932 and A20 cells. In U2932 cells, MnTE-2-PyP^{5+} specifically glutathionylated the p65 NF-κB subunit, suggesting that protein glutathionylation may be critical for the porphyrin's ability to induce cell death in these cells, which is like our

findings in the WEHI7.2 cell culture model. The mechanism by which glutathionylation inhibits NF-κB, however, remains unclear.

In human hepatoma cells, glutathionylation of p65 sequesters p65 in the cytoplasm [40]. Therefore, it is possible that in WEHI7.2 and U2932 cells, glutathionylation of the p65 NF-κB subunit by MnTE-2-PyP^{5+} inhibits its ability to translocate into the nucleus, and/or its ability to bind to DNA and activate transcription. Another possibility is that MnTE-2-PyP^{5+} glutathionylates the NF-κB that is already located in the nucleus and bound to DNA. Our results in the A20 B-cell lymphoma cells suggest that the porphyrin may glutathionylate NF-κB that is already located in the nucleus. The A20 cell line has an inactivating mutation in A20, a ubiquitin editing enzyme that results in constitutively active NF-κB [38]. Treatment with MnTE-2-PyP^{5+} increased protein glutathionylation in the A20 cells and in combination with dexamethasone, the porphyrin increased protein glutathionylation to an even greater extent. The porphyrin also decreased the number of viable cells and in combination with dexamethasone enhanced dexamethasone-induced cell death. Additional studies are needed to determine whether p65 glutathionylation inhibits NF-κB activity and promotes cell death in the A20 cells by disrupting DNA binding.

Previously, we also demonstrated that MnTE-2-PyP^{5+} must redox cycle with an oxidant such as H_2O_2 to induce cell death [32]. In this study, we found that in DLBCL and MM cells, dexamethasone and bortezomib increase H_2O_2 and that the porphyrin synergizes with these drugs. It is likely that in the presence of H_2O_2 the active site manganese in MnTE-2-PyP^{5+} is oxidized to the Mn(IV) state. When the manganese is oxidized to the Mn(IV) state, cell-free studies have shown that MnTE-2-PyP^{5+} redox cycles with small molecules like glutathione or cysteines to reduce the manganese from the Mn(IV) back to the Mn(III) state [19]. The oxidation

of glutathione and cysteines in proteins results in the oxidation of the redox environment and in the glutathionylation of proteins. This model suggests that the redox environment or concentration and type of oxidants in the cell affects the cells' vulnerability to MnTE-2-PyP^{5+}. Tumor cells with a high oxidant concentration are likely to be more susceptible to MnTE-2-PyP^{5+} as a single agent while others with a lower concentration would only be susceptible when treated with a compound or chemotherapeutic that increases oxidative stress.

The ability of the porphyrin to enhance cell death only in the presence of H_2O_2 or an oxidized redox environment provides a therapeutic window for treatment of tumors with MnTE-2-PyP^{5+} because of the relative difference between the oxidant load of normal and tumor cells. Several studies have shown that cancer cells are more oxidized than their normal counterparts [9, 10]. Previously, we showed that in peripheral mononuclear blood cells (PMBC), the normal counterpart of DLBCL, MnTE-2-PyP^{5+} does not decrease cell viability nor does it enhance dexamethasone-induced apoptosis [33]. In the current study, we have also shown that treatment with dexamethasone does not increase the levels of H_2O_2 in PMBCs. The results in PMBCs further highlight the importance of H_2O_2 for the porphyrin's ability to induce apoptosis and suggest that the ability of MnTE-2-PyP^{5+} to enhance cell death is specific to tumor cells.

Our results also suggest that MnTE-2-PyP^{5+} may synergize with other drugs that increase H_2O_2. N^1, N^{11}-bis(ethyl)nonspermine (BENSpm), a polyamine analogue, and vitamin C generate H_2O_2 [43, 44]. Both BENSpm and vitamin C have been evaluated in phase I and II clinical trials for breast, prostate and lung cancers [43, 44]. Combining MnTE-2-PyP^{5+} with BENSpm, vitamin C, or other agents that increase H_2O_2 would expand the number and types of cancer that could potentially benefit from MnTE-2-PyP^{5+} treatment.

If MnTE-2-PyP^{5+} is to be used clinically, it is also important to determine the effect it has with other chemotherapeutics used to treat hematologic malignancies. We found that MnTE-2-PyP^{5+} also synergizes with cyclophosphamide and vincristine in ABC DLBCL cells. Cyclophosphamide is normally bioactivated through hydroxylation by the cytochrome P450 system in the liver [45]. The hydroxylated cyclophosphamide released by the liver is thought to be responsible for the cytotoxic effects of cyclophosphamide [45]. In a cell-free system, MnTE-2-PyP^{5+} acts as a cytochrome P450 reductase mimetic and hydroxylates cyclophosphamide [45]. The potentiation of cyclophosphamide toxicity is consistent with MnTE-2-PyP^{5+} acting as a cytochrome P450 reductase mimetic in the U2932 cells. The mechanism by which MnTE-2-PyP^{5+} synergizes with vincristine in U2932 cells has yet to be investigated. In a lymphoblastoma cell culture model (HOB1 cells), vincristine has been shown to increase ROS [46]. The inhibition of ROS production by N-acetyl-L-cysteine, a ROS scavenger, moderately decreased vincristine-induced apoptosis in HOB1 cells [46]. Thus, it is possible that vincristine increases ROS in U2932 cells and provides the oxidized redox environment necessary for the porphyrin to enhance cell death.

Overall, our studies demonstrate that the manganese porphyrin, MnTE-2-PyP^{5+}, synergizes with drugs that are standard of care for DLBCL to enhance cell death in human DLBCL cell lines. Cells dependent on NF-κB were also sensitive to MnTE-2-PyP^{5+} alone. In MM cells, MnTE-2-PyP^{5+} synergizes with glucocorticoids and bortezomib to enhance cell death. In future studies, we hope to test the porphyrin in a lymphoma or MM animal model. These studies will help us determine whether the addition of MnTE-2-PyP^{5+} to the standard therapy for DLBCL and MM may overcome NF-κB-mediated survival, enhance cell death, and ultimately improve patient survival.

REFERENCES

1. Harris, N.L., et al., *The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997.* Mod Pathol, 2000. **13**(2): p. 193-207.
2. Alizadeh, A.A., et al., *Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling.* Nature, 2000. **403**(6769): p. 503-11.
3. Friedberg, J.W. and R.I. Fisher, *Diffuse large B-cell lymphoma.* Hematol Oncol Clin North Am, 2008. **22**(5): p. 941-52, ix.
4. Lichtman, M.A., *Battling the hematological malignancies: the 200 years' war.* Oncologist, 2008. **13**(2): p. 126-38.
5. Fisher, R.I., et al., *Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma.* N Engl J Med, 1993. **328**(14): p. 1002-6.
6. Fu, K., et al., *Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse large B-cell lymphoma.* J Clin Oncol, 2008. **26**(28): p. 4587-94.
7. Nickenig, C., et al., *Combined cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) improves response rates but not survival and has lower hematologic toxicity compared with combined mitoxantrone, chlorambucil, and prednisone (MCP) in follicular and mantle cell lymphomas: results of a prospective randomized trial of the German Low-Grade Lymphoma Study Group.* Cancer, 2006. **107**(5): p. 1014-22.
8. Alexanian, R., et al., *Primary dexamethasone treatment of multiple myeloma.* Blood, 1992. **80**(4): p. 887-90.

9. Wondrak, G.T., *Redox-directed cancer therapeutics: molecular mechanisms and opportunities.* Antioxid Redox Signal, 2009. **11**(12): p. 3013-69.

10. Trachootham, D., J. Alexandre, and P. Huang, *Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?* Nat Rev Drug Discov, 2009. **8**(7): p. 579-91.

11. Boonstra, J. and J.A. Post, *Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells.* Gene, 2004. **337**: p. 1-13.

12. Szatrowski, T.P. and C.F. Nathan, *Production of large amounts of hydrogen peroxide by human tumor cells.* Cancer Res, 1991. **51**(3): p. 794-8.

13. Briehl, M.M., et al., *Mitochondria and redox homoeostasis as chemotherapeutic targets.* Biochem Soc Trans, 2014. **42**(4): p. 939-44.

14. Tome, M.E., M.C. Jaramillo, and M.M. Briehl, *Hydrogen peroxide signaling is required for glucocorticoid-induced apoptosis in lymphoma cells.* Free Radic Biol Med, 2011. **51**(11): p. 2048-59.

15. Bera, S., et al., *Dexamethasone-induced oxidative stress enhances myeloma cell radiosensitization while sparing normal bone marrow hematopoiesis.* Neoplasia, 2010. **12**(12): p. 980-92.

16. Wang, S., et al., *Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms. intermediacy of H(2)O(2)- and p53-dependent pathways.* J Biol Chem, 2004. **279**(24): p. 25535-43.

17. Trevino, A.V., et al., *Enhanced topoisomerase II targeting by annamycin and related 4-demethoxy anthracycline analogues.* Mol Cancer Ther, 2004. **3**(11): p. 1403-10.

18. Gewirtz, D.A., *A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin.* Biochem Pharmacol, 1999. **57**(7): p. 727-41.

19. Batinic-Haberle, I., J.S. Reboucas, and I. Spasojevic, *Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential.* Antioxid Redox Signal, 2010. **13**(6): p. 877-918.

20. Gardner, P.R., D.D. Nguyen, and C.W. White, *Superoxide scavenging by Mn(II/III) tetrakis (1-methyl-4-pyridyl) porphyrin in mammalian cells.* Arch Biochem Biophys, 1996. **325**(1): p. 20-8.

21. Pasternack, R.F., et al., *Catalysis of the disproportionation of superoxide by metalloporphyrins. III.* J Inorg Biochem, 1981. **15**(3): p. 261-7.

22. Ferrer-Sueta, G., et al., *Reduction of manganese porphyrins by flavoenzymes and submitochondrial particles: a catalytic cycle for the reduction of peroxynitrite.* Free Radic Biol Med, 2006. **41**(3): p. 503-12.

23. Day, B.J. and C. Kariya, *A novel class of cytochrome P450 reductase redox cyclers: cationic manganoporphyrins.* Toxicol Sci, 2005. **85**(1): p. 713-9.

24. Spasojevic, I., et al., *New approach to the activation of anti-cancer pro-drugs by metalloporphyrin-based cytochrome P450 mimics in all-aqueous biologically relevant system.* J Inorg Biochem, 2006. **100**(11): p. 1897-902.

25. Batinic-Haberle, I., I. Spasojevic, and I. Fridovich, *Tetrahydrobiopterin rapidly reduces the SOD mimic Mn(III) ortho-tetrakis(N-ethylpyridinium-2-yl)porphyrin.* Free Radic Biol Med, 2004. **37**(3): p. 367-74.

26. Moeller, B.J., et al., *A manganese porphyrin superoxide dismutase mimetic enhances tumor radioresponsiveness.* Int J Radiat Oncol Biol Phys, 2005. **63**(2): p. 545-52.

27. Moeller, B.J., et al., *Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules.* Cancer Cell, 2004. **5**(5): p. 429-41.

28. Tse, H.M., M.J. Milton, and J.D. Piganelli, *Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigen-presenting cells: implication for their use in targeting oxidation-reduction reactions in innate immunity.* Free Radic Biol Med, 2004. **36**(2): p. 233-47.

29. Jackson, I.L., et al., *Superoxide dismutase mimetic reduces hypoxia-induced O2*-, TGF-beta, and VEGF production by macrophages.* Free Radic Res, 2007. **41**(1): p. 8-14.

30. Spasojevic, I., et al., *Pharmacokinetics of the potent redox-modulating manganese porphyrin, MnTE-2-PyP(5+), in plasma and major organs of B6C3F1 mice.* Free Radic Biol Med, 2008. **45**(7): p. 943-9.

31. Crow, J.P., et al., *Manganese porphyrin given at symptom onset markedly extends survival of ALS mice.* Ann Neurol, 2005. **58**(2): p. 258-65.

32. Jaramillo, M.C., et al., *Manganese porphyrin, MnTE-2-PyP5+, Acts as a pro-oxidant to potentiate glucocorticoid-induced apoptosis in lymphoma cells.* Free Radic Biol Med, 2012. **52**(8): p. 1272-84.

33. Jaramillo, M.C., et al., *Increased manganese superoxide dismutase expression or treatment with manganese porphyrin potentiates dexamethasone-induced apoptosis in lymphoma cells.* Cancer Res, 2009. **69**(13): p. 5450-7.

34. Jaramillo, M.C., et al., *Manganese (III) meso-tetrakis N-ethylpyridinium-2-yl porphyrin acts as a pro-oxidant to inhibit electron transport chain proteins, modulate bioenergetics, and enhance the response to chemotherapy in lymphoma cells.* Free Radic Biol Med, 2015. **83**: p. 89-100.

35. Kabe, Y., et al., *Redox regulation of NF-kappaB activation: distinct redox regulation between the cytoplasm and the nucleus.* Antioxid Redox Signal, 2005. **7**(3-4): p. 395-403.

36. Davis, R.E., et al., *Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells.* J Exp Med, 2001. **194**(12): p. 1861-74.

37. Hideshima, T., et al., *NF-kappa B as a therapeutic target in multiple myeloma.* J Biol Chem, 2002. **277**(19): p. 16639-47.

38. Lee, E.G., et al., *Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice.* Science, 2000. **289**(5488): p. 2350-4.

39. Chou, T.C. and P. Talalay, *Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors.* Adv Enzyme Regul, 1984. **22**: p. 27-55.

40. Qanungo, S., et al., *Glutathione supplementation potentiates hypoxic apoptosis by S-glutathionylation of p65-NFkappaB.* J Biol Chem, 2007. **282**(25): p. 18427-36.

41. Jain, D., et al., *Cardiac complications of cancer therapy: Pathophyisiology, identificationn, prevention, treatment, and future directions.* Curr Cardiol Rep, 2017. **19**(5):36.

42. Brown, C.O., et al., *Interleukin-6 counteracts therapy-induced cellular oxidative stress in multiple myeloma by up-regulating manganese superoxide dismutase.* Biochem J., 2012. **443**(3):515-27.

43. Pledgie A., et. al., *Spermine oxidase SMO(PAOh1), Not N1-acetylpolamine oxidase PAO, is the primary source of cytotoxic H2O2 in polyamine analogue-treated human breast cancer cell lines.* J Biol Chem. 2005. **280**(48):39843-39851.

44. Chen Q., et al., *Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues.* Proc Natl Acad Sci USA. 2005. **102**(38):13604-13609.

45. Spasojevic I., et al., *New approach to the activation of anti-cancer pro-drugs by metalloporphyrin-based cytochrome P450 mimics in all-aqueous biologically relevant system.* J Inorg Biochem. 2006. **100**: 1897-1902.

46. Tsai, S.Y., et al., *Involvement of reactive oxygen species in multidrug resistance of a vincristine-selected lymphoblastoma*. Cancer Sci. 2007. **98**(8):1206-14.