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Chemical Modulation of Cancer Cells to Enhance Tumor Immunity

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Abstract

Breakthroughs in immunotherapy have led to cancer therapeutics that activate the immune system by blocking inhibitory mechanisms. This class of therapeutics has resulted in longer survival rates for cancer patients, some living over 10 years after being diagnosed as terminally ill. However, only a small fraction of patients who receive immunotherapy drugs respond favorably. Recent studies suggest that certain anticancer agents (both cytotoxic chemotherapeutics and targeted drugs) stimulate immune recognition of cancers. Identification of such immunomodulatory anticancer agents would make ideal partners for current immunotherapy treatments, thus increasing the proportion of the treated patients who benefit from immunotherapy. This study aimed to identify the immunomodulatory effects of several anticancer agents by using a novel cell co-culture assay that modeled recognition of breast cancer cells by natural killer cells (NK cells). This physiologically relevant assay was used to systematically characterize effects of anticancer drugs on NK cell function. This assay successfully identified four types of immunomodulatory effects: antagonistic (suppressive), synergistic (enhancing), additive, and no effect on NK cell function. Further investigation confirmed the modulatory effects of those compounds that had been identified as antagonistic or synergistic.

Introduction

Cancer immunotherapy is an emerging field of cancer research, which focuses on developing therapeutics that target the immune system instead of cancer cells. Tumor cells develop immune resistance by taking advantage of inhibitory checkpoint mechanisms. Normally these inhibitory mechanisms are used to prevent the immune system from attacking healthy tissue (autoimmunity). However, cancer cells can cause dysregulation in the expression of inhibitory 'checkpoint' proteins and this can lead to immune resistance. These types of checkpoint mechanisms rely on ligand-receptor interactions and can be blocked by therapeutic antibodies.[1] Among the first such inhibitory receptors to be identified were the Cytotoxic T-lymphocyte antigen 4 (CTLA-4) and Programmed Death 1 (PD-1) receptors. Development of therapeutic antibodies to block these receptors essentially removes the brakes on the immune system and allows the immune system to mount an attack on cancer cells.

A large focus in this field is identifying checkpoint receptors and developing antibody treatments that help activate tumor-cancer recognition, such as Bristol Myers Squibb's anti-CTLA-4 antibody treatment, ipilimumab, for metastatic melanoma.[2] Treatment with immunotherapy therapeutics has produced impressive long term survival of some cancer patients, some living several years after being diagnosed with advanced stages of cancer. However only about 15-20% of treated patients benefit from immunotherapy treatment.[3,4,5,6] For example, ipilimumab only produced long term survival beyond two years in 18% of patients in one clinical trial.[1] A promising solution to increasing the proportion of patients who benefit from treatment is to use immunotherapy in combination with other anti-cancer agents.[7] A study on 502 previously untreated metastatic melanoma patients found that long term survival beyond 3 years was occurring in 20.8% of patients who were assigned to take ipilimumab along with dacarbazine (chemotherapy drug) versus only 12.2% long term survival in patients only receiving dacarbazine.[4]

The goal of this study is to identify potential anti-cancer agents that enhance cancer-immune recognition and could be prioritized for used in combination with current cancer immunotherapy treatments. Another goal is to identity which anti-cancer agents suppress cancer-immune recognition, in order to deprioritize them as potential combination partners. Immune-cancer interactions will be modeled using a novel cell co-culture assay that models recognition of breast cancer cells (MDA-MB-231) by natural killer cells (NK cells; a type of effector immune cells). It is predicted that treatment of cancer cells with anti-cancer agents can alter their destruction by natural killer cells *in vitro*. NK cell activity can be extrapolated from growth inhibition of GFP+ MDA cells when compared to GFP+ MDA cells grown alone. This will allow a better understanding of what immunomodulatory effects anti-cancer agents have on the innate immune system.

Methods

Tissue Culture

Breast Cancer Cells

The MDA-MB-231 cell line that overexpresses a green fluorescent protein (GFP) construct was cultured in Dulbecco's modified Eagle medium enriched with 10% fetal bovine serum and 1% pen strep. Cells were incubated at 37°C and 5% CO_2.

Natural Killer Cells

Peripheral Blood CD56+ CD16+ Natural Killer Cells, Negative Selection (Lonza, Walkersville, MD) were cultured in LGM-3™ Lymphocyte Growth Medium (Lonza, Walkersville MD) supplemented with 10% Fetal Bovine Serum. Interleukin-2 (IL-2) (PeproTech, Rocky Hill, NJ) was added to NK cell culture at a concentration of 100 units per mL.

High Throughput Screening of Anti-Cancer Agents on MDA NK Cell Co-Culture

Breast cancer cells from cell line MDA-MB-231 were plated in corning black with clear flat bottom 348 well assay plates (Corning Inc., Corning, NY) at a density of 1000 cells per well with a total volume of 50µL, and incubated overnight at 37°C and 5% CO_2. The next day compounds were added to the plates using a CyBi-Well Bario 384/60 µL Head (CyBio, Woburn, MA) and 100nL of common anti-cancer agent was added to each well. Anti-cancer agents were obtained from the compound set previously used for the NCI-CTD^2 project (http://www.broadinstitute.org/ctrp.v2/); see Table 3 (in supplemental material) for compounds. The stock concentration for the compounds varied, see Table 3 (in supplemental material) for concentration details. Plates were incubated at 37°C and 5% CO_2 overnight. The following day, compound-containing media was removed and natural killer cells (experimental) or LGM3 + IL2 media alone (control) were added at a ratio of 12.5 to 1, NK cells to MDA cells. Assay plates were then incubated for the next 6 days at 37°C and 5% CO_2 and imaged daily.

Dose Response Series

Forty selected compounds were serially diluted to 0.333X, 0.111X and 0.0370X the initial concentration (see Table 5 in supplemental material for compounds and detailed concentrations). Breast cancer cells (MDA-MB-231) were plated in 384 well assay plates the same as previously described. After cells adhered overnight, the compounds were added using the same protocol as before. After a 24hour compound treatment, compound media was removed and natural killer cells (experimental) or LGM3 + IL2 (control) were added at a ratio of 12.04 to 1, NK cells to MDA cells. Assay plates were then incubated as described previously.

Testing ABT-737's Synergistic Mechanism

Compounds ABT-737, ABT-199 and SZ4TA2 were obtained from the same CTD^2 collection as before. Initial stock concentration of ABT-737 was 10mM, and was diluted to 5mM in DMSO. Initial stock concentrations of ABT-199 and SZ4TA2 were both 20mM and both compounds were diluted to 2.5mM in DMSO. Cancer cells were plated in 384 well assay plates the same as previously described. After an overnight incubation period, compounds were added as previously described. After a 24 h compound treatment, compound media was removed and NK cells (experimental) or LGM3 + IL2 (control) were added at a ratio of 12 to 1, NK cells to MDA cells. Assay plates were then incubated the same as described before.

Microscopy Imaging

High content microscopy (IXMicro, Molecular Devices) was used to image 384 well plates. Cells were imaged under GFP wavelengths and bright field illumination in order to track MDA cell growth (GFP+ cells) and NK cell activity. Microscope magnification was set at 4X. For the GFP wavelength, exposure was set at 200ms and target max intensity at 50000. For the bright field (transmitted light) wavelength, exposure was set at 200ms, target max intensity at 3000 and Z-offset from GFP was -50µm. Cells were imaged for 6 days (one-time point per day) starting on the day compounds were added. Cells were quantified using MetaXpress software, using a 'Count Nuclei' algorithm. Parameters for this analysis were set at approximate min width 10µm = 6 pixels, approximate max width 20µm = 12 pixels and set to an intensity above local background of 300 graylevels.

Results

Global overview of Compounds Screened in MDA-NK Cell Co-Culture Assay

The immunomodulatory effects of many of the compounds tested in this study have already been identified (see Table 1-3). Importantly, this study further confirms their immunomodulatory effects and validates this co-culture assay as a tool that can correctly predict immunomodulatory effects. As proof of concept, this study was successfully able to identify the immunomodulatory effects of 320 anti-cancer agents (Table 4). Of the 320 compounds screened in the MDA-NK cell culture assay more than half (51.56%) of the compounds were manually identified to be additive, which can be seen in Figure 1. Under this condition the compounds caused a partial reduction in cancer cell growth and this effect combined with the natural killer cells abilities created an additive effect that was the sum of the combined effects. Another observed effect was antagonism which accounted for 12.81% of the compounds screened. Compounds in this group exhibited an immunosuppressive effect i.e. the combined cancer inhibitory effect of the compound and NK cells was less than the cancer inhibitory effect of NK cells alone. The synergistic group of compounds which consisted of 4.69% of the tested compounds, displayed immune enhancing effects, i.e. the combined cancer inhibitory effect of compound and NK cells was far greater than the effects of compound or NK cells alone. The last category to be observed was the group of compounds that had no effect on natural killer cell function and consisted of 30.94% of the compounds screened.

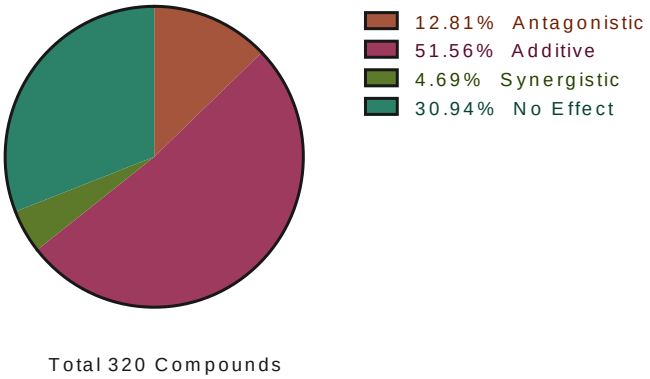

Figure 1. Global Overview of Immunomodulatory Effects on 320 Screened Compounds. Compounds were categorized by manual inspection into four groups: antagonistic, additive, synergistic and having no effect on NK cell activity. Antagonistic compounds exhibited an immunosuppressive effect, while synergistic compounds exhibited immune enhancing effects. Additive compounds demonstrated a combined effect from the compound and NK cells that resulted in a total effect that equaled the sum of the two effects. No effect compounds matched the controls indicating no effect on NK cell activity.

Natural Killer Cells Successfully Inhibit Cancer Cell Growth

When NK cells were co-cultured along with cancer cells, there was an observable difference when compared to cancer cells cultured alone. NK cells cause a reduction in cancer cell growth when compared to the growth of cancer cells alone (Figure 2A). This indicates that NK cells are capable of killing cancer cells *in vitro*, without the presence of anti-cancer agents. These results were visually confirmed by comparing the bright field and GFP channel images that correspond to (Figure 2B). In the control condition's GFP images, there's an increase in GFP+ cells with each day, which is also reflected in the bright field images. Meanwhile, in the NK cells alone condition's GFP images, there is a reduction of GFP+ cells, indicating cancer cell growth inhibition. In the bright field there is a visible formation of clumps of cancer cells that are surrounded and attacked by NK cells**.**

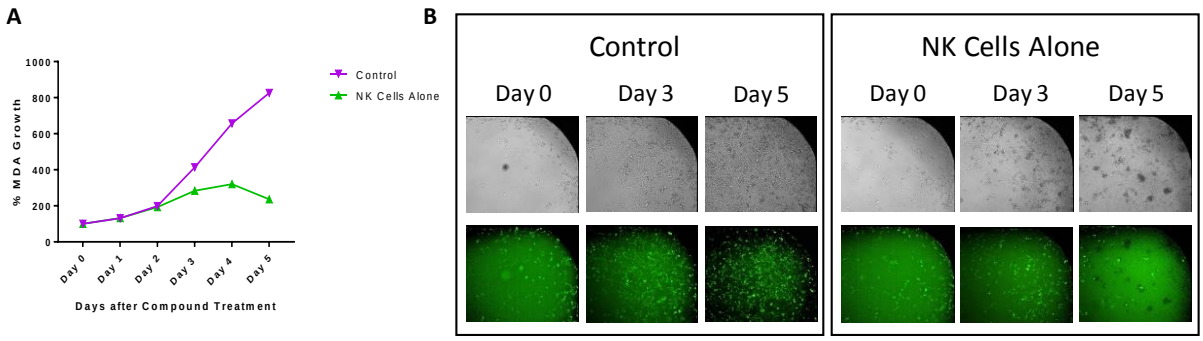

Figure 2. Effects of NK cells on GFP+ Breast Cancer Cells. Two controls were tracked across 6 days, one with only cancer cells (control) and one with cancer cells and NK cells (NK cells alone). These represent the effects of NK cells on breast cancer cells in a single assay without the presence of compounds. (A) Cancer cell growth was normalized to Day 0 (indicating initial density at 100%) and means were graphed.(B) Bright-field illumination (top row) and GFP fluorescent illumination illustrate the effects of NK cells on cancer cell proliferation.

Identifying the Immunomodulatory Effects Anti-Cancer Agents Have on NK Cell Activity

When pre-treated with compound and co-cultured with NK cells, four effects on cancer cell growth were observed (Figure 3). Pifithrin-alpha (Figure 3A) is an example of a compound with no effect on natural killer cell function. In this figure the condition with compound only matches up closely with the control line, and the NK cell + compound combination condition also matches up with the NK cell only condition, indicating that this compound had no effect on Natural killer cell activity. MK-2206 (Figure 3B) is an example of an antagonistic immune suppressive compound. While the compound alone doesn't decrease cancer cell growth, in combination with NK cells cancer growth is greater compared to the NK only condition. This suggests that MK-2206 is immune suppressive. Erlotinib (Figure 3C) is an example of an additive compound. In combination with NK cells, erlotinib causes a greater growth suppression of cancer cells compared to the NK cell alone condition. This indicates that Erlotinib combined with NK cells produces an additive effect, which is the sum of the effects of the compound and NK cells on cancer cells. ABT-737 (Figure 3D) exhibits effects that are synergistic and immune enhancing. When the compound alone condition is compared to the control condition, there is a similar amount of cancer growth. However, in combination with NK cells there is a dramatic decrease in cancer cell growth when compared to the NK cell alone condition. This suggests that this compound was synergistic with immune cells in mediating cancer cell suppression.

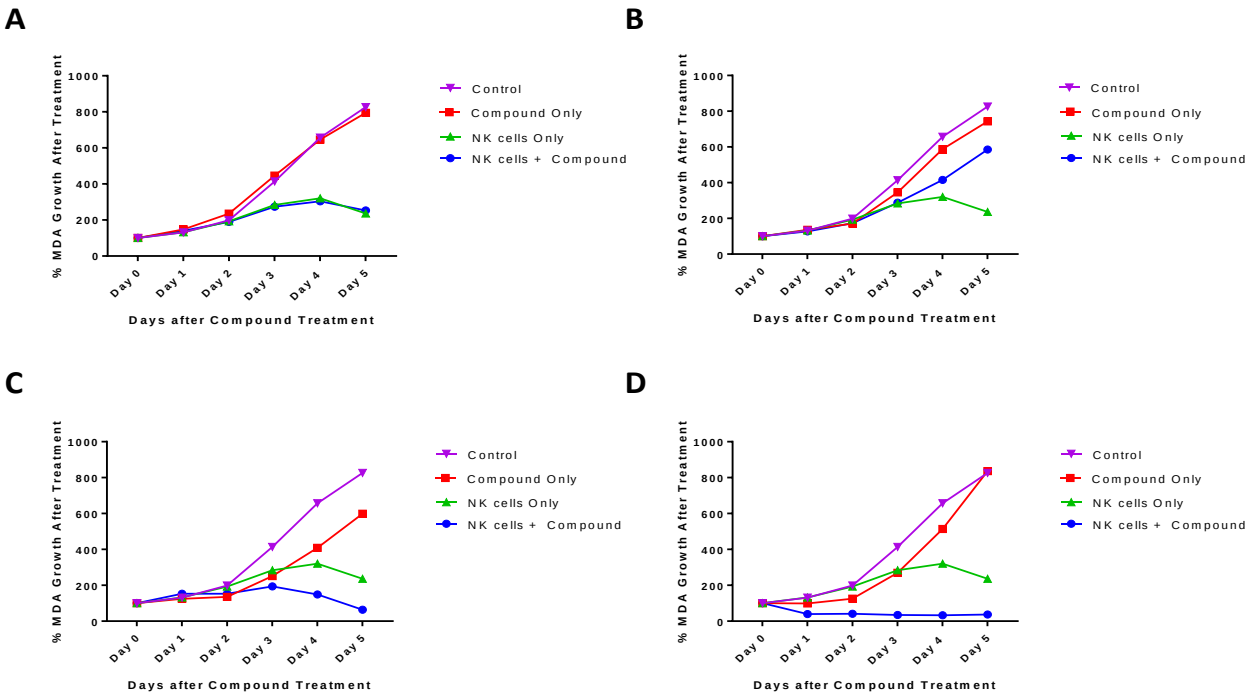

Figure 3. Immunomodulatory Effects of Anti-Cancer Agents on Natural Killer Cells. Breast cancer cells were pre-treated with compounds for 24 hours (Day 0) before introducing natural killer cells. The four types of observed immunomodulatory effects are illustrated by (A) Pifithrin-Alpha – no effect, (B) MK-2206 – antagonstics effect, (C) Erlotinib – additive effect, and (D) ABT-737 – synergistic effect. Each line represents the mean growth of MDA-MB-231 breast cancer cells normalized to Day 0. Each compound was tested under four conditions: a control with no NK cells and no compound, a compound only condition with no NK cells, a NK cells only condition with no compound, and a combination condition with both NK cells and compound.

Microscopy Images Confirm Immunomodulatory Effects

Daily images (GFP and brightfield) were taken for 5 days after 24 h compound pre-treatment at each time interval for each compound. Figure 4 shows the images that correspond to treatments presented in Figure 3. Figure 4 compares the NK cells only condition with the NK + compound combination condition. Treatment with pifithrin-alpha leads to a similar decrease of GFP+ cells when compared to treatment with NK cells only. This supports the conclusion that pifithrin-alpha had no effect on the ability of NK cells to kill cancer cells (Figure 4A). Figure 4B verifies the immunosuppressive effects of MK-2206, where it can be seen that NK cells + MK-2206 leads to an increase of GFP+ cells compared to treatment with NK cells alone. Erlotinib's additive effects on cancer cell growth are illustrated in Figure 4C, where the combination of erlotinib with NK cells leads to a reduction in cancer cell growth compared to NK cells alone. Note that in the bright field images for this drug a pronounced clumping of cancer cells that is absent in the NK cell only treatment. A strong synergistic effect is illustrated for ABT-737 in combination with NK cells on inhibition of cancer cell growth (Figure 4D). There was a striking decrease in GFP+ cancer cells in this treatment that is far more pronounced than in any other treatments presented, indicating that ABT-737 is highly immune enhancing.

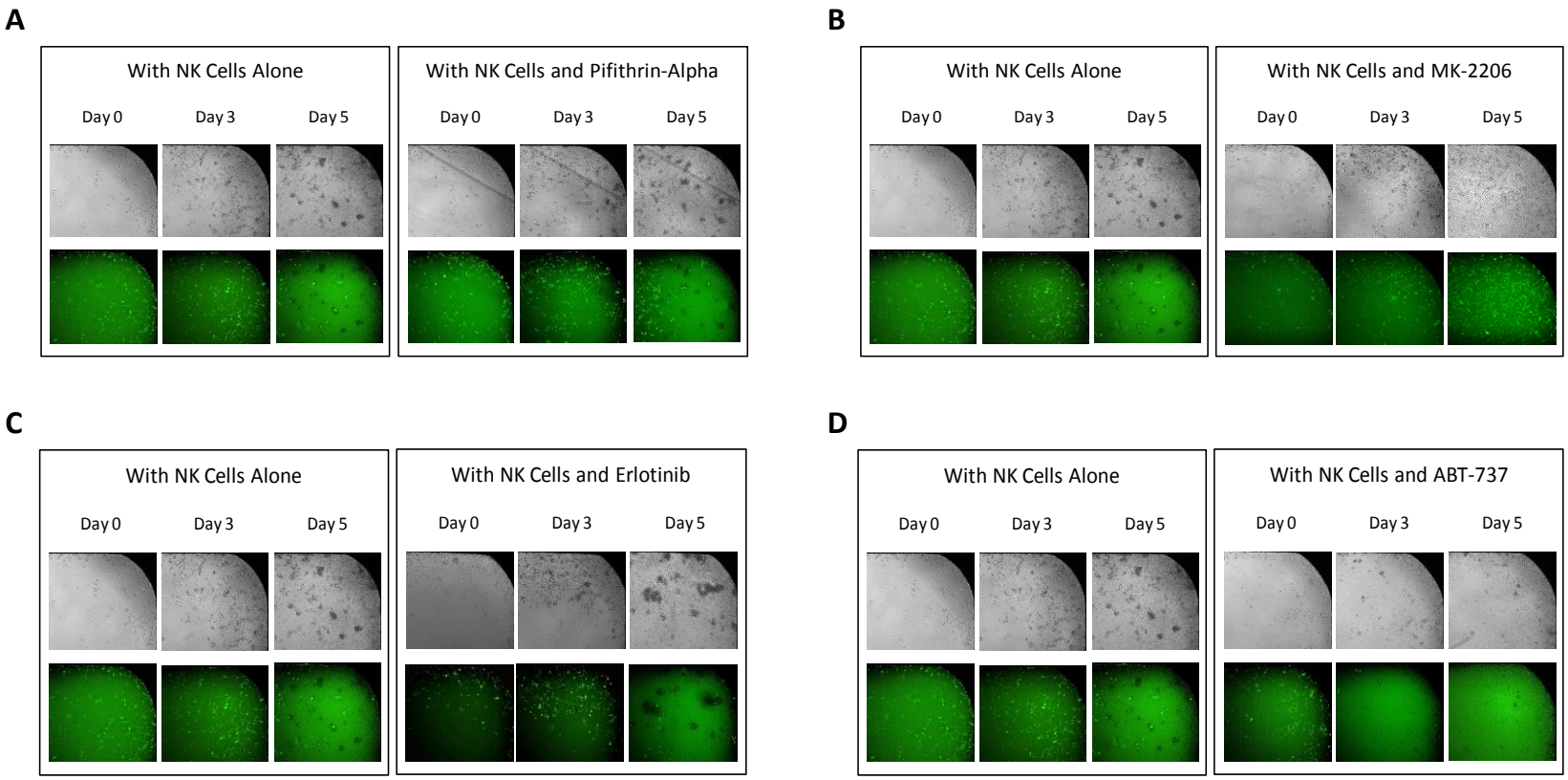

Figure 4. Imaging Visually Confirms Immunomodulatory Effects of Screened Compounds. All compound conditions were imaged under bright-field (top row) and GFP (bottom row) wavelengths. Each compound (A) Pifithrin-Alpha, (B) MK-2206, (C) Erlotinib and (D) ABT-737 visually displayed what was observed in the cancer growth graphs: (A) no effect, (B) antagonistic, (C) additive and (D) synergistic. All compounds are compared to NK alone control condition.

Dose Response Series Verifies Immune Enhancing and Immune Suppressive Effects

Select compounds were tested in a dose response series, where their modulatory effects were observed at different concentrations. Cells were pre-treated with compound and imaged the same as in the initial drug screen. Figure 5 shows the dose response for two compounds that had been previously seen to be either enhancing or suppressing immune activity. MK-2206 (Figure 5A), which was immunosuppressive in the initial drug screen, was found to be immunosuppressive at all concentrations when compared to the DMSO control with no compound (0 µM concentration condition). This confirms MK-2206's immunosuppressive effects towards NK cell activity. ABT-737 (Figure 5B) was previously seen to be immune enhancing and synergistic in the initial drug screen, and this effect was confirmed across various concentrations when compared to the DMSO control. Although these compounds were able to demonstrate their modulatory effects at all concentrations, they were most effective at higher concentrations.

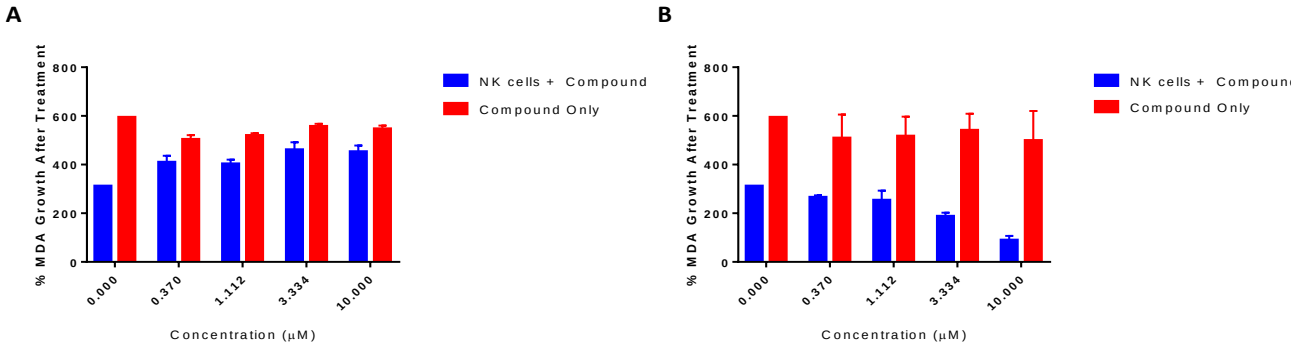

Figure 5. Dose Response Series Confirms Immunomodulatory Effects. The modulatory effect of (A) MK-2206 – immunosuppressive, and (B) ABT-737 – immune enhancing, were confirmed across a variety of concentrations. The concentration of 0.000 µM is the DMSO control. Red bars represent growth of cancer cells in the presence of the compound, while blue bars represent the combined effects of NK cells and the compounds.

Exploring Synergistic Mechanism of ABT-737

ABT-737 is an inhibitor of several proteins in the BCL family, including BCL-2, BCL-xl and BCL-w. In order to investigate the potential mechanism by which ABT-737 is synergistic, ABT-199 and SZ4TA2 (BCL selective compounds) were tested alongside ABT-737. ABT-199 is a selective inhibitor of BCL-2, and SZ4TA2 is a selective inhibitor of BCL-xL. While the effects of ABT-737 in this trial were similar to previous experiments (compare Figure 3D, 6A), neither of the other BCL family inhibitors exhibited this synergistic effect (Figure 6B, C). This indicates that both ABT-199 and SZ4TA2 have no effect on natural killer cell activity.

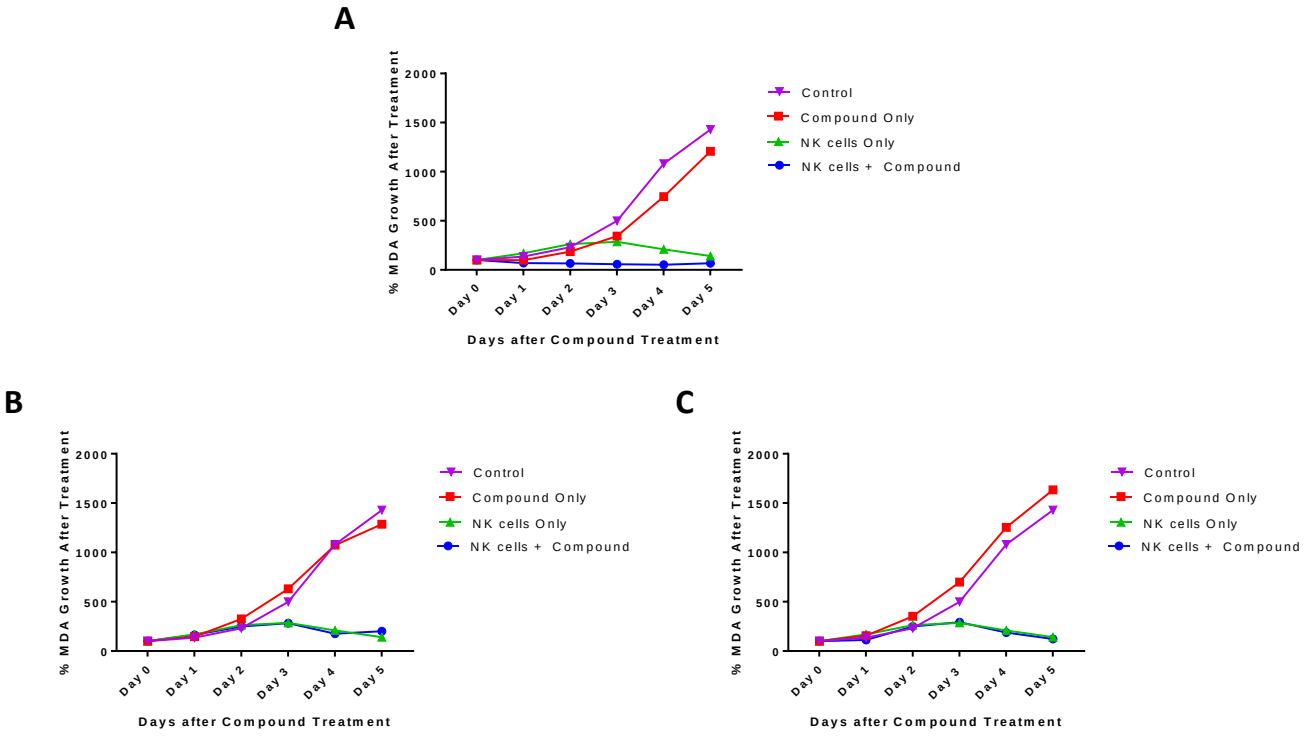

Figure 6. Investigating ABT-737's Synergistic Mechanism. Cells were pre-treated for 24 hours before adding NK cells. (A) ABT-737 (10µM) which inhibits BCL-2, BCL-xl and BCL-W was compared to two compounds that selectively inhibit certain proteins in the BCL-2 family. (B) ABT-199 (5µM) is selectively inhibits BCL-2, while (C) SZ4TA2 (5µM) selectively inhibits BCL-xL. Synergistic effects of ABT-737 were confirmed, while both ABT-199 and SZ4AT2 showed no effect.

Discussion

We were successfully able to determine that treatment of cancer cells with anti-cancer agents does alter their destruction by natural killer cells *in vitro*. Some of these compounds such as erlotinib are already in clinical trials as combination partners with current immunotherapy treatments (www.clinicaltrials.gov, identifier NCT02013219). In addition to identifying known immunomodulatory effects, this study was able to identify many unknown immunomodulatory effects. Compounds identified as synergistic or immune enhancing (4.69%) should be given priority when considering potential immunotherapy combination partners. While compounds that were identified as antagonistic or immune suppressive (12.81%) should be deprioritized when considering potential immunotherapy combination partners. This study also identified most of the

compounds as additive (51.56%). However, further investigation into these compounds is required to determine if they would make good combination partners with immunotherapy.

Another important aspect that this study uncovered was successfully modeling cancer immune interactions *in vitro*. This study confirmed that NK cells are able to destroy breast cancer cells *in vitro*. As a result of this ability, we were able to use this assay to determine the immunomodulatory effects of anti-cancer agents. This study was successfully able to identify four types of immunomodulatory effects: antagonistic, synergistic, additive, and no effect on NK cell activity. Examples of such identified compounds include MK-2206 (antagonistic), ABT-737 (synergistic), erlotinib (additive), and pifithrin-alpha (no effect). And a dose response series of select compounds was able to confirm their immunomodulatory effects. For example, the effect of MK-2206 as immunosuppressive was confirmed at all tested concentrations, and the immune enhancing effect of ABT-737 was also confirmed. Despite demonstrating immunomodulatory effects at low concentrations, the compounds all seemed to be most effective at higher concentrations. Some additional aspects that need to be considered in future studies are the effects of the tumor microenvironment; specifically, the immunosuppressive effects of the tumor microenvironment. In order to make this assay more physiologically relevant, immunosuppressive cells (such as stromal cells) would need to be introduced, and the effects of the compounds would need to be monitored. Other types of cancer cells would also need to be tested in order to see if these immunomodulatory effects are specific to breast cancer or if they can by generalized to other types of cancer.

By identifying immunomodulatory effects, we can make new hypotheses on mechanisms that make compounds immunosuppressive or immune enhancing. One such mechanism that we further investigated was the mechanism that made ABT-737 synergistic. ABT-737 is a compound that inhibits BCL-2, BCL-xL and BCL-W, and the BCL-2 protein family is associated with regulating cell death. Dysregulation or overexpression of the BCL-2 family can inhibit cell death pathways and lead to malignancies.[8] Therefore inhibiting BCL-2 family proteins has the potential to stimulate apoptosis under certain conditions and thus prevent potential cancerous or pre-cancerous cells from becoming malignant. In order to determine which member of the BCL-2 family was causing synergism, two selective compounds were tested side by side to ABT-737. ABT-199 is a selective inhibitor of BCL-2 and SZ4TA2 is a selective inhibitor of BCL-xL. It was found that neither of these compounds had any immunomodulatory effects, indicating that BCL-2 and BCL-xL alone do not cause synergistic effects. This suggests that synergism may either be caused by BCL-W or by combining the inhibitory effects of two or all three of these BCL-2 family proteins. Further studies would need to combine compounds and introduce a BCL-W selective inhibitor in order to fully determine the mechanism that makes ABT-737 synergistic.

Conclusion

This study confirms the usefulness of a co-culture assay of NK cells and cancer cells as an *in vitro* method to screen potential anti-cancer agents. We successfully identified certain compounds as suppressive or enhancing which will be useful for prioritizing and deprioritizing immunotherapy combination partners. The usefulness of this *in vitro* assay was illustrated by confirming previously known modulatory effects such as erlotinib's additive effect that resulted in greater reduction of cancer cell growth or survival. In addition, the assay identified previously unknown effects such as MK-2206's suppressive effects and ABT-737's enhancing effects on NK cell activity. Despite these promising findings, further research is needed to finish testing all the known anti-cancer agents, to see the effects that the tumor microenvironment has on anti-cancer agent's immunomodulatory effects and to see if these effects are specific to breast cancer cells or if they can be generalized to more cancers.

Acknowledgements

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Citations

[1] Pardoll, Drew M. "The blockade of immune checkpoints in cancer immunotherapy." *Nature Reviews Cancer* 12.4 (2012): 252-264.

[2] Couzin-Frankel, Jennifer. "Cancer immunotherapy." *Science* 342.6165 (2013): 1432-1433.

[3] Robert, Caroline, et al. "Ipilimumab plus dacarbazine for previously untreated metastatic melanoma." *New England Journal of Medicine* 364.26 (2011): 2517-2526.

[4] Brahmer, Julie R., et al. "Safety and activity of anti–PD-L1 antibody in patients with advanced cancer." *New England Journal of Medicine* 366.26 (2012): 2455-2465.

[5] Borghaei, Hossein, et al. "Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer." *New England Journal of Medicine*373.17 (2015): 1627-1639.

[6] Robert, C., Schachter, J., Long, G. V., Arance, A., Grob, J. J., Mortier, L., ... & Larkin, J. (2015). Pembrolizumab versus ipilimumab in advanced melanoma. *New England Journal of Medicine*, *372*(26), 2521-2532.

[7] Galluzzi, Lorenzo, et al. "The secret ally: immunostimulation by anticancer drugs." *Nature reviews Drug discovery* 11.3 (2012): 215-233.

[8] Yip, K. W., and J. C. Reed. "Bcl-2 family proteins and cancer." *Oncogene*27.50 (2008): 6398-6406.

Tables

[9] Nars, Mariana S., and Ramon Kaneno. "Immunomodulatory effects of low dose chemotherapy and perspectives of its combination with immunotherapy." International Journal of Cancer 132.11 (2013): 2471-2478.

[10] Markasz, Laszlo, et al. "Effect of frequently used chemotherapeutic drugs on the cytotoxic activity of human natural killer cells." Molecular cancer therapeutics 6.2 (2007): 644-654.

[11] Bose, Anamika, et al. "Combined vaccine+ axitinib therapy yields superior anti-tumor efficacy in a murine melanoma model." *Melanoma research* 22.3 (2012): 236.

[12] Wei, Jun, et al. "Topotecan enhances immune clearance of gliomas." *Cancer immunology, immunotherapy* 58.2 (2009): 259-270.

[13] Drake, C. G. "Combination immunotherapy approaches." *Annals of oncology*23.suppl 8 (2012): viii41-viii46.

[14] Ramakrishnan, Rupal, et al. "Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice."*The Journal of clinical investigation* 120.4 (2010): 1111-1124.

[15] Hannesdóttir, Lára, et al. "Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response." *European journal of immunology*43.10 (2013): 2718-2729.

[16] Morrissey, Kari M., et al. "Immunotherapy and Novel Combinations in Oncology: Current Landscape, Challenges, and Opportunities." *Clinical and translational science* 9.2 (2016): 89-104.

[17] Micheau, Olivier, et al. "Sensitization of cancer cells treated with cytotoxic drugs to fas-mediated cytotoxicity." *Journal of the National Cancer Institute*89.11 (1997): 783-789.

[18] Liu, Ji-Yan, et al. "Single administration of low dose cyclophosphamide augments the antitumor effect of dendritic cell vaccine." *Cancer Immunology, Immunotherapy* 56.10 (2007): 1597-1604.

[19] Brittenden, Julie, et al. "Natural killer cells and cancer." *Cancer* 77.7 (1996) : 1226-1243.

[20] Wang, Y., et al. "Temsirolimus, an mTOR inhibitor, enhances anti-tumour effects of heat shock protein cancer vaccines." *British journal of cancer*104.4 (2011): 643-652.

[21] De Vecchis, L., et al. "Amplification of natural killer activity of mouse lymphocytes by vincristine." *International journal of tissue reactions* 4.4 (1981): 283-289.

[22] Koido, Shigeo, et al. "Current immunotherapeutic approaches in pancreatic cancer." *Clinical and Developmental Immunology* 2011 (2011).

[23] Davies, Faith E., et al. "Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma." *Blood* 98.1 (2001): 210-216.

[24] He, Sisi, et al. "Enhanced interaction between natural killer cells and lung cancer cells: involvement in gefitinib-mediated immunoregulation." *Journal of translational medicine* 11.1 (2013): 1.

[25] West, Alison C., et al. "An intact immune system is required for the anticancer activities of histone deacetylase inhibitors." *Cancer research*73.24 (2013): 7265-7276.

[26] Sagiv-Barfi, Idit, et al. "Ibrutinib enhances the antitumor immune response induced by intratumoral injection of a TLR9 ligand in mouse lymphoma."*Blood* 125.13 (2015): 2079-2086.

[27] Lowe, Devin B., et al. "Dasatinib promotes the expansion of a therapeutically superior T-cell repertoire in response to dendritic cell vaccination against melanoma." *Oncoimmunology* 3.2 (2014): e27589.

[28] Salih, Julia, et al. "The BCR/ABL-inhibitors imatinib, nilotinib and dasatinib differentially affect NK cell reactivity." *International Journal of cancer* 127.9 (2010): 2119-2128.

[29] Heine, A., et al. "Immunomodulatory effects of anti-angiogenic drugs."*Leukemia* 25.6 (2011): 899-905.

[30] Boccadoro, Mario, Gareth Morgan, and Jamie Cavenagh. "Preclinical evaluation of the proteasome inhibitor bortezomib in cancer therapy." *Cancer cell international* 5.1 (2005): 1.

[31] Nelson, Robert P., and Mark Ballow. "26. Immunomodulation and immunotherapy: drugs, cytokines, cytokine receptors, and antibodies."*Journal of allergy and clinical immunology* 111.2 (2003): S720-S732.

[32] Matsue, Hiroyuki, et al. "Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and dexamethasone) on bidirectional dendritic cell-T cell interaction during antigen presentation." *The Journal of Immunology* 169.7 (2002): 3555-3564.

[33] Kodumudi, Krithika N., et al. "A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers." *Clinical Cancer Research* 16.18 (2010): 4583-4594.

[34] Salih, Julia, et al. "The BCR/ABL-inhibitors imatinib, nilotinib and dasatinib differentially affect NK cell reactivity." *International Journal of Cancer* 127.9 (2010): 2119-2128.

Supplemental Material

Table 1. Common Immune Enhancing Anti-Cancer Agents

Compound	Effects	Source
Etoposide*	Enhances NK cell activity	Nars et al., 2013 Markasz et al., 2007
Axitinib*	Enhances activation and recruitment of $CD8^+$ T cells	Bose et al., 2012
Topotecan*	Enhances immune tumor recognition via Fas signaling	Wei et al., 2009
Dacarbazine	Improves overall survival when combined with immune checkpoint therapy	Drake et al., 2012
Doxorubicin*	Increases IL-12 expression by Dendritic cells Enhances T cell activity Enhances NK cell activity	Nars et al., 2013 Ramakrishnan et al., 2010 Hannesdóttir et al., 2013 Markasz et al., 2007
Cisplatin	In clinical trials for combination therapy with immune checkpoint inhibitor Shown to sensitize tumors to Fas mediated cytotoxicity	Morrissey et al., 2016 Micheau et al., 1997
Cyclophosphamide	Augments antitumor immune response of Dendritic Cells Suppressive to immune system at high doses (augments immune response at low doses)	Liu et al., 2007 Brittenden et al., 1996
Trametinib	In clinical trials with BRAF inhibitor and PD1 inhibitor combination therapy	Morrissey et al., 2016
Temsirolimus	Increases interferon-γ and T-cell response when used in combination with cancer vaccines	Wang et al., 2011
Lapatinib	Enhances interferon-γ secreting T-cells present at tumor site when used in	Hannesdóttir et al., 2013

	combination with Doxorubicin	
Vincristine*	Enhances NK cell activity	De Vecchis et al., 1981
Pazopanib*	In clinical trials for combination therapy with immune checkpoint inhibitor	Morrissey et al., 2016
Gemcitabine*	Inhibits myeloid suppressor cells but doesn't reduce CD4+ T cells, CD8+ T cells, NK cells, macrophages, or B cells	Koido et al., 2011
Erlotinib*	In clinical trials for combination therapy with immune checkpoint inhibitor	Morrissey et al., 2016
Thalidomide*	Enhances NK cell numbers and activity	Davies et al., 2001
Gefitinib*	Enhances NK cell Activity	He et al., 2013
Vorinostat*	Enhances expansion of IFNg-producing T-cells	West et al., 2013
Ibrutinib*	Immune response enhanced when used in combination with a TLR9 ligand	Sagiv-Barfi et al., 2015

** Assay identified known immune modulatory effects*

Table 2. Common Immune Suppressive Anti-Cancer Agents

Compound	Effects	Source
Dasatinib	Has been seen to be immune enhancing	Lowe et al., 2014
		Salih et al., 2010
	However, it is suppressive to NK cell activity	Heine et al., 2011
Bortezomib	Shown to enhance antitumor properties	Boccadoro et al., 2011
	However, it is suppressive to NK cell activity	Markasz et al., 2007
Methotrexate	Suppressive to NK cell activity	Brittenden et al., 1996
		Nelson et al., 2003
	Inhibits macrophage activation	
Paclitaxel	Shown to inhibit tumor growth, and recruit CD4+ and CD8+ T cell	Nars et al., 2013
		Ramakrishnan et al., 2010
	However, it is suppressive to T-cell and NK cell activity	Markasz et al., 2007
Dexamethasone*	Inhibits dendritic cell maturation	Matsue et al., 2002
Docetaxel	Enhances T-cell activity	Kodumudi et al., 2010

	However it is suppresses NK cell activity	Markasz et al., 2007

* *Assay identified known immune modulatory effects*

Table 3. Common Anti-Cancer Agents with No Immunomodulatory Effects

Compound	Effects	Source
Mitomycin	NK cell activity is not affected when used in combination with other chemotherapy agents	Brittenden et al., 1996
Nilotinib*	NK cell cytotoxicity is not altered, but NK cytokine production is decreased at high concentrations	Salih et al., 2010

* *Assay identified known immune modulatory effects*

Table 4. Immunomodulatory Effects of CTD² Compound Collection Subset

Compound Name	Stock Concentration mM	Final Concentration µM	Modulatory Effect
CAY10576	0.1	0.2	No Effect
UNC0638	5	10	Additive
Bafilomycin A1	0.1	0.2	Additive
Etoposide	5	10	Additive
Narciclasine	0.1	0.2	Additive
SRT-1720	5	10	No Effect
SB-743921	0.1	0.2	Additive
JW-480	5	10	No Effect
Dasatinib	5	10	No Effect
BRD-K34485477	5	10	No Effect
SN-38	0.1	0.2	Additive
BRD-K01737880	5	10	Additive
Bortezomib	0.1	0.2	No Effect
BRD-K44224150	5	10	No Effect
Brefeldin A	0.1	0.2	Additive
Axitinib	5	10	Additive
BRD-A96645632	5	10	No Effect
Triazolothiadiazine	5	10	Additive
SGX-523	0.1	0.2	Additive
GW-843682X	5	10	No Effect
Barasertib	5	10	Additive
ML239	5	10	Additive
SU11274	5	10	No Effect
PF-573228	5	10	Additive

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
SCH-79797	5.5	11	No Effect
Afatinib	5	10	Additive
AZD8055	5	10	Additive
Doramapimod	5	10	Additive
BRD-K34222889	5	10	Additive
GDC-0941	5	10	Antagonistic
Itraconazole	5	10	No Effect
CAL-101	5	10	Antagonistic
Topotecan	5	10	Additive
CHIR-99021	5	10	No Effect
Methotrexate	5	10	No Effect
XL765	5	10	Synergistic
Compound 7d-cis	5	10	Additive
Spautin-1	5	10	Synergistic
PD318088	5	10	Antagonistic
Dacarbazine	5	10	Antagonistic
SR8278	0.1	0.2	No Effect
Paclitaxel	5	10	Additive
Mitomycin	5	10	Additive
Neratinib	5	10	Additive
BRD4132	25	50	Additive
Vorapaxar	5	10	Additive
ML006	25	50	Synergistic
Tipifarnib	5	10	Additive
RAF265	5	10	Antagonistic
Veliparib	5	10	No Effect
Serdemetan	5	10	No Effect
AT13387	5	10	Additive
MST-312	5	10	Additive
AZD7762	5	10	Additive
KU 0060648	5	10	Antagonistic
Doxorubicin	5	10	Additive
BRD-K63431240	5	10	Additive
ML029	5	10	No Effect
BRD-K04800985	0.1	0.2	Additive
Tamatinib	5	10	Additive
Teniposide	5	10	No Effect
Semagacestat	5	10	Additive
CAY10618	5	10	Antagonistic

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
NSC19630	5	10	Additive
Pifithrin-mu	5	10	Additive
Lenvatinib	5	10	Additive
WZ4002	5	10	Additive
BRD-K66453893	5	10	Additive
QS-11	5	10	No Effect
BRD-K96970199	5	10	No Effect
Tanespimycin	5	10	Additive
BRD-K33199242	5	10	Synergistic
Cediranib	5	10	Synergistic
BRD-K41597374	5	10	No Effect
Trifluoperazine	5	10	No Effect
BRD-K50799972	5	10	No Effect
Alvocidib	5	10	No Effect
GSK-J4	5	10	Additive
Cytarabine Hydrochloride	5	10	Additive
Masitinib	5	10	No Effect
BRD-K27224038	0.1	0.2	Antagonistic
Manumycin A	5	10	Additive
ISOX	5	10	Additive
PD 153035	5	10	Additive
AT7867	5	10	Additive
ML162	5	10	No Effect
Repligen 136	5	10	Additive
BRD-K92856060	5	10	Additive
Cisplatin	5	10	Antagonistic
SJ-172550	5	10	Antagonistic
SZ4TA2	5	10	No Effect
AC55649	5	10	Synergistic
Tozasertib	5	10	Additive
BRD-K09344309	5	10	No Effect
PF-3758309	5	10	Additive
CCT036477	5	10	Synergistic
Methazolastone	5	10	Additive
HLI 373	5	10	Additive
ML258	0.1	0.2	No Effect
PF-184	5	10	Additive
Clofarabine	5	10	Additive

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
Necrosulfonamide	5	10	No Effect
WZ8040	5	10	No Effect
NSC48300	5	10	Additive
Nintedanib	5	10	No Effect
BRD-K19103580	5	10	Additive
Compound 1541A	5.5	11	Additive
BRD-K41334119	5	10	Synergistic
PI-103	5.5	11	No Effect
MI-2	5	10	No Effect
CHM-1	4.8	9.6	Additive
JW-55	5	10	No Effect
PAC-1	5	10	No Effect
BRD-K13999467	5	10	No Effect
Apicidin	5	10	Additive
BRD-K17060750	5	10	Antagonistic
WP1130	5	10	No Effect
Bexarotene	5	10	Antagonistic
GSK-3 inhibitor IX	5	10	Additive
SMER-3	5	10	No Effect
Dinaciclib	0.1	0.2	Additive
Daporinad	5	10	No Effect
BRD-K34099515	5	10	Additive
VER-155008	5	10	Additive
Salermide	25	50	Additive
BMS-536924	5	10	Additive
Cyclophosphamide	0.1	0.2	No Effect
MK-2206	5	10	Antagonistic
BCL-LZH-4	0.1	0.2	No Effect
BIX-01294	5	10	Additive
Navitoclax	5	10	Additive
AZD1775	5	10	Additive
ML203	5	10	Additive
Ciclosporin	5	10	No Effect
Indisulam	5	10	No Effect
CD-437	5	10	Additive
Vandetanib	5	10	No Effect
Tivozanib	5	10	Additive
Dexamethasone	0.1	0.2	Antagonistic
Obatoclax	5	10	Antagonistic

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
LY-2183240	5	10	Additive
MGCD-265	5	10	Additive
NVP-BSK805	5	10	Additive
Importazole	25	50	No Effect
Nilotinib	5	10	No Effect
Tamoxifen	25	50	No Effect
Vorinostat	5	10	Additive
N9-isopropylolomoucine	25	50	Additive
Oligomycin A	5	10	Antagonistic
Fumonisin B1	25	50	Antagonistic
Belinostat	5	10	Additive
AM-580	25	50	Antagonistic
BRD-K33514849	5	10	Antagonistic
Necrostatin-1	25	50	No Effect
Cyanoquinoline 11	5	10	Antagonistic
Zebularine	25	50	Antagonistic
Cytochalasin B	5	10	Additive
Valdecoxib	25	50	Antagonistic
TW-37	5	10	No Effect
BRD8958	5	10	No Effect
Panobinostat	0.1	0.2	Additive
Gefitinib	5	10	Additive
QW-BI-011	5	10	Additive
NVP-ADW742	5	10	No Effect
BRD-K42260513	5	10	Additive
Alisertib	5	10	Additive
BRD6340	5	10	Additive
Parbendazole	5	10	Additive
SCH-529074	5	10	Additive
AZ-3146	5	10	Additive
PLX-4720	5	10	No Effect
FGIN-1-27	5	10	No Effect
ML210	5	10	Additive
Erastin	5	10	Additive
YK 4-279	5	10	Additive
MLN2238	5	10	Additive
Bax channel blocker	5	10	Antagonistic
BRD-K02251932	5	10	Synergistic

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
Omacetaxine Mepesuccinate	0.1	0.2	Additive
STF-31	5	10	No Effect
ABT-737	5	10	Synergistic
Mdivi-1	5	10	Additive
Bardoxolone Methyl	5	10	No Effect
Neuronal Differentiation Inducer III	25	50	Additive
Crizotinib	5	10	Additive
BIBR-1532	25	50	Synergistic
Foretinib	5	10	No Effect
PF-750	25	50	Additive
Trametinib	5	10	No Effect
CI-976	25	50	Synergistic
Tacedinaline	5	10	Antagonistic
Cimetidine	25	50	Antagonistic
Bosutinib	5	10	Additive
Etomoxir	25	50	No Effect
Piperlongumine	5	10	Additive
Sildenafil	25	50	Synergistic
KX2-391	5	10	Additive
Phloretin	25	50	No Effect
Silmitasertib	5	10	Additive
COL-3	5	10	No Effect
Leptomycin B	0.1	0.2	Additive
PF-543	5	10	Additive
Epigallocatechin-3-monogallate	25	50	Additive
Temsirolimus	5	10	Antagonistic
BI-2536	0.1	0.2	Additive
NSC632839	5	10	No Effect
Myriocin	0.1	0.2	Additive
R428	5	10	Antagonistic
ABT-199	5	10	No Effect
Lapatinib	5	10	Antagonistic
Canertinib	5	10	Additive
Erismodegib	5	10	Additive
BRD-K04574331	5	10	Synergistic
PRIMA-1-Met	5	10	No Effect

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
I-BET151	5	10	No Effect
NSC95397	5	10	No Effect
WAY-362450	5	10	No Effect
GMX-1778	5	10	Additive
Sepantronium Bromide	0.1	0.2	No Effect
BRD-K37390332	5	10	Antagonistic
Merck60	5	10	Additive
Regorafenib	5	10	Additive
Pluripotin	5	10	Additive
NSC23766	25	50	Additive
AZD4547	5	10	Additive
ATRA	25	50	Additive
GSK1059615	5	10	Additive
SKI-II	25	50	Additive
Cabozantinib	5	10	Additive
Purmorphamine	25	50	Antagonistic
Fedratinib	5	10	No Effect
PDMP	25	50	Additive
Niclosamide	5	10	Additive
Pifithrin-alpha	25	50	No Effect
NVP-TAE684	5	10	Additive
MI-1	0.1	0.2	Antagonistic
BRD9647	5	10	No Effect
UNC0321	0.1	0.2	No Effect
Fingolimod	5	10	No Effect
BMS-195614	5	10	No Effect
Dactolisib	0.1	0.2	Additive
Tubastatin A	5	10	Additive
BEC	5	10	Additive
Isoliquiritigenin	5	10	Additive
9-dihydropiplartine	5	10	Additive
Istradefylline	5	10	Synergistic
AA-COCF3	5	10	Additive
AGK-2	5	10	Additive
Curcumin	5	10	No Effect
Tipifarnib-P1	5	10	Antagonistic
GW-405833	5	10	No Effect
Ko-143	25	50	Additive

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
PRIMA-1	5	10	Additive
Isoevodiamine	5	10	Additive
Olaparib	5	10	Additive
SNX-2112	5	10	Additive
Lovastatin	5	10	Additive
Abiraterone	5	10	No Effect
SM-406	0.1	0.2	Additive
CD-1530	25	50	Additive
GDC-0879	5	10	No Effect
KU-60019	5	10	Additive
Lomeguatrib	5	10	Additive
Docetaxel	0.1	0.2	Additive
CID-5951923	5	10	Additive
BRD-K14844214	0.1	0.2	Additive
Betulinic acid	5	10	Additive
Vincristine	0.1	0.2	Additive
Hyperforin	5	10	Additive
Ouabain	0.1	0.2	No Effect
Pazopanib	5	10	Additive
Blebbistatin	0.1	0.2	Additive
NVP-231	5	10	Additive
BRD-K96431673	0.1	0.2	No Effect
KU-0063794	5	10	Additive
LY-2157299	0.1	0.2	No Effect
Sirolimus	5	10	Antagonistic
ML312	0.1	0.2	Antagonistic
1S	5	10	No Effect
OSI-027	5	10	No Effect
Avicin D	5	10	No Effect
HMN-214	5	10	Additive
AZD7545	5	10	Additive
Rigosertib	5	10	Additive
BRD-K88742110	5	10	Additive
SID 26681509	25	50	Additive
ML320	5	10	Additive
BMS-270394	5	10	No Effect
SR1001	5	10	No Effect
PRL-3 inhibitor I	25	50	Antagonistic
Darinaparsin	5	10	No Effect

Table 4. Immunomodulatory Effects of CTD^2 Compound Collection Subset			
Compound Name	**Stock Concentration mM**	**Final Concentration µM**	**Modulatory Effect**
Nutlin-3	5	10	No Effect
PF-4800567	5	10	Antagonistic
Simvastatin	5	10	No Effect
KW-2449	5	10	Additive
TGX-221	5	10	No Effect
Ruxolitinib	5	10	Antagonistic
BMS-345541	5	10	Additive
Gemcitabine	0.1	0.2	Additive
SNS-032	5	10	Additive
Ch-55	5	10	No Effect
ML204	5	10	No Effect
Nelarabine	5	10	Additive
Ifosfamide	0.1	0.2	No Effect
Sotrastaurin	5	10	Antagonistic
BRD-K09587429	0.1	0.2	Additive
Erlotinib	5	10	Additive
Thalidomide	0.1	0.2	Additive
Ibrutinib	5	10	Additive
BRD-K29086754	0.1	0.2	No Effect
Ki8751	5	10	Additive
BRD-K49290616	0.1	0.2	Antagonistic
BRD1812	5	10	Additive
Fulvestrant	0.1	0.2	No Effect
AZD1480	5	10	Antagonistic
CBB-1007	0.1	0.2	No Effect
Necrostatin-7	5	10	No Effect
O-6-benzylguanine	0.1	0.2	No Effect
Isonicotinohydroxamic acid	5	10	Additive
Azacitidine	5	10	Antagonistic

Table 5. Immunomodulatory Effects of Compounds Screened in Dose Response Series			
Compound	**Stock Concentrations**	**Final Concentrations**	**Modulatory Effect**
Erlotinib	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
I-BET151*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Additive
Olaparib*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Additive

Table 5. Immunomodulatory Effects of Compounds Screened in Dose Response Series			
Compound	**Stock Concentrations**	**Final Concentrations**	**Modulatory Effect**
KU-0063794	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
Lovastatin	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Synergistic
Panobinostat*	0.10mM, 0.033mM, 0.011mM, 0.0037mM	0.20µm, 0.066µM, 0.022µM, 0.0074µM	Additive
Neuronal Differentiation Inducer III*	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00µM, 16.666µM, 5.556µM, 1.852µM	Additive
Vorapaxar	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
NVP-BSK805	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Antagonistic
Doramapimod	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
Tipifarnib	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Antagonistic
BRD-K66453893*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Additive
PF-750	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00µM, 16.666µM, 5.556µM, 1.852µM	Synergistic
Navitoclax	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Synergistic
GSK-3 Inhibitor IX*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Additive
Compound 1541A*	5.50mM, 1.833mM, 0.611mM, 0.204mM	11.00µM, 3.666µM, 1.222µM, 0.408µM	Additive
SCH-529074	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
Temsirolimus*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Antagonistic
Trametinib*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Antagonistic
Oligomycin A	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
BRD-K27224038	0.10mM, 0.033mM, 0.011mM, 0.0037mM	0.20µm, 0.066µM, 0.022µM, 0.0074µM	No Effect
ABT-737*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Synergistic
ML006	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00µM, 16.666µM, 5.556µM, 1.852µM	No Effect
BRD-K41334119	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
BRD-K66453893	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	No Effect
BRD-K04574331*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00µM, 3.334µM, 1.112µM, 0.370µM	Synergistic

Table 5. Immunomodulatory Effects of Compounds Screened in Dose Response Series			
Compound	**Stock Concentrations**	**Final Concentrations**	**Modulatory Effect**
Spautin-1	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	No Effect
Istradefylline	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	No Effect
Sildenafil	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00μM, 16.666μM, 5.556μM, 1.852μM	No Effect
KU 0060648*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	Antagonistic
BRD-K37390332	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	No Effect
MK-2206*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	Antagonistic
R428*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	Antagonistic
AM-580*	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00μM, 16.666μM, 5.556μM, 1.852μM	Antagonistic
Cyanoquinoline	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	No Effect
MI-1	0.10mM, 0.033mM, 0.011mM, 0.0037mM	0.20μm, 0.066μM, 0.022μM, 0.0074μM	No Effect
Bexarotene*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	Antagonistic
Dexamethasone*	0.10mM, 0.033mM, 0.011mM, 0.0037mM	0.20μm, 0.066μM, 0.022μM, 0.0074μM	Antagonistic
Ruxolitinib*	5.00mM, 1.667mM, 0.556mM, 0.185mM	10.00μM, 3.334μM, 1.112μM, 0.370μM	Antagonistic
PRL-3 Inhibitor I	25.00mM, 8.333mM, 2.778mM, 0.926mM	50.00μM, 16.666μM, 5.556μM, 1.852μM	No Effect

**Serial dose response verified modulatory effect*