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TITLE: Understanding Drug Resistance to Targeted Therapeutics in Malignant B-Cell Lymphoproliferative Disorders

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14. ABSTRACT: Small molecule inhibitors of B cell receptor (BCR) mediated signaling broadened therapeutic alternatives for patients with B-cell lymphoproliferative disorders (B-LPDs), but understanding of drug resistance mechanisms is needed. We studied correlative samples from 15 subjects enrolled on the clinical trial of the novel microenvironmentally targeting combination of lenalidomide and plerixafor. We discovered increasing longitudinal CXCR4 expression as a possible mechanism enabling cells to migrate along the CXCR4-CXCL12 axis to the protective niches of the microenvironment. Surface CD52 (decreases in those remaining on therapy), and soluble APRIL (increased in a subject previously progressing on a PI3Kdelta inhibitor) may be biomarkers to risk for primary or secondary resistance. We also studied primary CLL cells exposed to tyrosine kinase inhibitors of interest including those that target BTK, PI3K, and MEK; and to caspase inhibitors as a marker of apoptotic machinery. Surprisingly caspase inhibition induced CLL cell death, rather than protecting, and does not appear that the mechanism for this finding is necroptosis. Finally we established co-culture stromal systems and flow based assays of drug induced apoptosis or death to evaluate the selected therapeutic inhibitors. Our ED50 and 50% reduction in target p-protein lowering may be due to patient selection. Ongoing experiments are detailed to confirm and expand these findings.						
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Table of Contents

	<u>Page(s)</u>
1. Introduction.....	1
2. Keywords.....	1
3. Overall Project Summary.....	1-15
4. Key Research Accomplishments.....	15
5. Conclusion.....	15-17
6. Publications, Abstracts, and Presentations.....	17
7. Inventions, Patents and Licenses.....	17
8. Reportable Outcomes.....	17
9. Other Achievements.....	17
10. References.....	18
11. Appendices Listing.....	21

Understanding Drug Resistance to Targeted Therapeutics in Malignant B-Cell Lymphoproliferative Disorders (B-LPDs)

AWARD NUMBER: W81XWH-13-1-0358

I. INTRODUCTION: For patients with relapsed or refractory B-cell lymphoproliferative disorders (B-LPDs), small molecule inhibitors of B cell receptor (BCR) mediated signaling including recently FDA approved inhibitors of Bruton's tyrosine kinase (ibrutinib; Imbruvica, Pharmacyclics) [1] and Pi3K delta (idelalisib; Zydelig, Gilead) [2], have broadened therapeutic alternatives with high rates of lymph node regression among heavily pretreated patients [3-8]. However, bone marrow responses are often delayed and incomplete with other patients failing to demonstrate any significant disease improvement (primary resistance), and another minority with progression on therapy (acquired/secondary resistance). Understanding the mechanisms of resistance specific to the novel small molecule inhibitors based on the drug target and important contributions of the microenvironment is critically needed. Given the specificity of the drug targets and their continuous method of administration, our central hypothesis is that drug resistance to pathway inhibitors is mediated by novel, target-specific, and microenvironment dependent mechanisms. The first aim is to explore these mechanisms for resistance in vitro and ex vivo in CLL correlative samples from patients treated in a phase I clinical trial targeting the microenvironment with lenalidomide and plerixafor and (2) in primary CLL patient derived peripheral malignant cells; the second aim is to define the role of the microenvironment in drug resistance to the targeted small molecule inhibitors through the utilization of stromal cell co-culture systems. Identification of specific mechanisms through these research goals can aid clinically to avoid complications of resistance, and to rationally design combinatory drug strategies that can improve the depth and duration of response from these promising therapeutic agents.

II. KEYWORDS:

- B-cell lymphoproliferative disorder (B-LPD)
- drug resistance
- Chronic Lymphocytic Leukemia/Small lymphocytic Lymphoma (CLL/SLL)
- microenvironment
- B-cell receptor (BCR)
- C-X-C chemokine receptor type 4 (CXCR-4/CD184)
- lenalidomide
- plerixafor
- tyrosine kinase inhibitors (TKIs)
- ibrutinib
- Phosphoinositide 3-kinase inhibitor (PI3K inhibitor)
- Mitogen/Extracellular signal-regulated kinase inhibitor (MEK inhibitor)
- Bruton's Tyrosine Kinase (BTK)
- apoptosis
- a proliferation-inducing ligand (APRIL)
- stromal co-cultures

III. OVERALL PROJECT PROGRESS SUMMARY: As a second year faculty member with aspirations for a career as an independent academic cancer research

clinician focusing on the indolent B cell lymphoproliferative disorders (B-LPDs), the CDMRP Visionary Postdoctoral Fellowship Award has provided a tremendous support mechanism for successful advancement toward these goals. Through this awarded project, we have made important progress in understanding the mechanisms of resistance specific to targeting the microenvironment of B-LPDs, and to the promising therapeutic small molecule inhibitors in various stages of clinical use. The knowledge generated from this project is applicable to preventative strategies that avoid complications of drug resistance, and to designing rational combinatory regimens that enhance and prolong therapeutic responses; ultimately working to decrease the impact of cancer on our patients. The experiences possible because of this funding enabled me to gain important skills in various laboratory techniques and data analysis. It also has provided invaluable protected time to delve into my proposal as well as establish relationships with new, diverse mentors and potential future collaborators who are vital to my career development and success.

Herein detail is provided on the specific experimental methods, results and troubleshooting, and conclusions based on the approved statement of work; and the summary of how this will provide a foundation for the second and third year of the proposal.

A. TASK 1: Obtain Regulatory Approval from local IRBs and the DoD Office of Research Protection.

1. Duke University Institutional Review Board and the DoD Office of Research

Protection: The subject protocol was approved by the Duke University Institutional Review Board (IRB) on 30 April 2013 with an expiration date current of 29 April 2015. This protocol was subsequently reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements; the official copy of this memo is housed with the protocol file at the Office of Research Protections, Human Research Protection Office, 810 Schreider Street, Fort Detrick, MD 21702-5000. An amendment/modification (P00001) for change of mentor to Dr. Daphne Friedman was approved with an effective date of 27 February 2014.

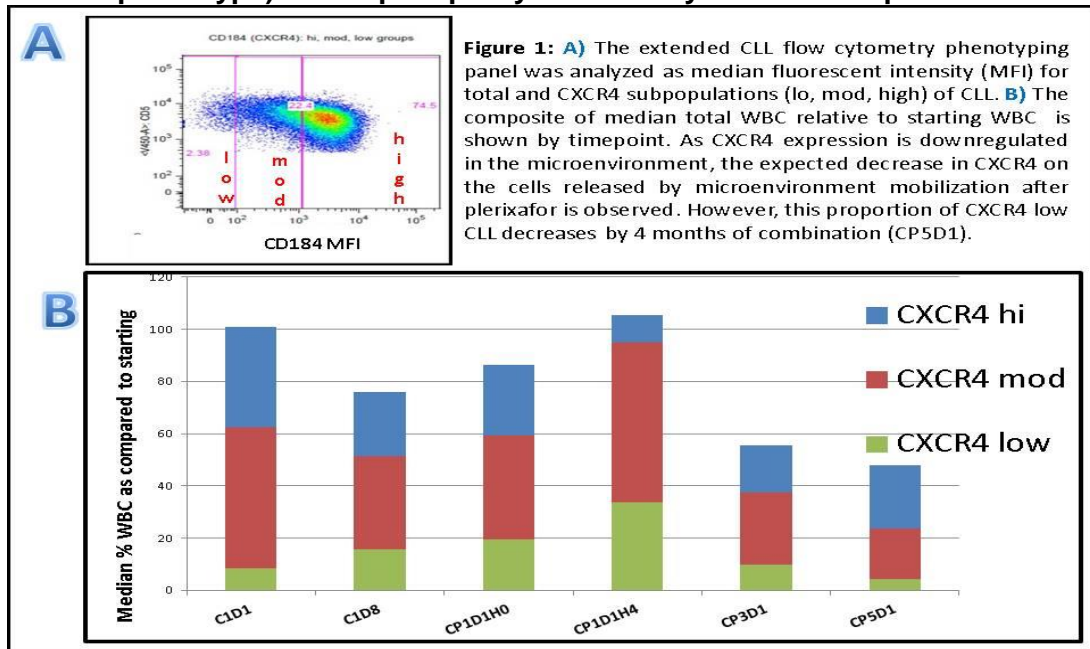
2. Durham VA Medical Institutional Review Board: The proposal was submitted to the VA IRB on 7 July 2014; and was placed in process pending the continuing review of the parent VA protocol for collection of subject primary peripheral blood CLL cells: The Chronic Lymphocytic Leukemia Research ID 01032, Prom# 0020 (PI Weinberg, collaborator). This study had a continuing review with approval on 9/29/14, contingent upon minor stipulations in language. Once formally approved, work can resume on approval of this proposal, award number W81XWH-13-1-0358.

B. TASK 2: Determine Mechanisms for resistance to the small molecule inhibitors *in vitro* and *ex vivo*:

The drugs that were selected for study included (1) inhibitors critical intracellular signaling pathways in B-LPD; this includes inhibitors of Phosphoinositide 3-kinase inhibitor (PI3K), Bruton's Tyrosine Kinase (BTK), and MEK1/2; and (2) the combination of lenalidomide and plerixafor as in the phase I clinical trial (NCT01373229) described in Appendix B. Lenalidomide is an immunomodulatory small molecule with efficacy in CLL, though monotherapy responses are typically partial and delayed. Plerixafor, a small molecule inhibitor of the CXCR4 receptor responsible for homing CLL to the microenvironment, has been safely utilized in CLL. The combination of plerixafor with lenalidomide offered a novel non-cytotoxic alternative to improve anti-tumor activity through targeting of the essential pro-survival microenvironment.

The focus of Task 2 during the 2013-2014 reporting period was on study of the clinical trial and the correlative samples collected at 7 preplanned timepoints along the treatment course as indicated by the red arrows in Appendix A, Figure 1. A total of 15 subjects were enrolled, with 10 initiating the combination of lenalidomide and plerixafor, 7 completing at least one cycle of the combination, and 6 completing 4 cycles of combination; all subjects are now off treatment phase of the trial and therefore sample collection is completed. The following refers to analysis of the correlatives for mechanisms of resistance per the aims of the SOW, except where otherwise stated.

1. Subtask #1: Identify selective differential signaling through pathways involved in proliferation and/or disruption of apoptotic pathways, as evaluated by changes in the surface expression of critical markers (surface flow cytometry immunophenotype) and in phosphorylation of key intracellular proteins:

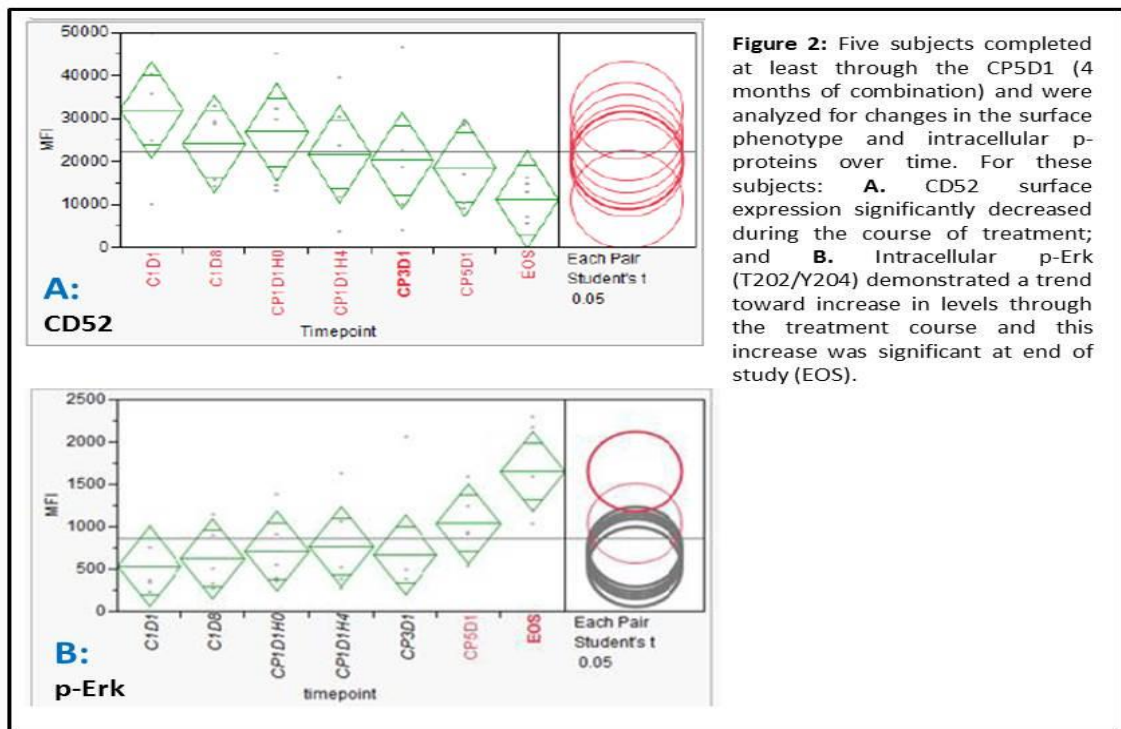


Methods: Blood was collected throughout study conduct for analyses including an extended CLL phenotyping by flow cytometry with analysis of CLL subpopulations by CXCR4 expression levels. The CLL cells are enriched from the blood using negative selection by RosetteSep (Stem Cell Technologies, Vancouver, Canada, and this yields $\geq 95\%$ CLL cell purity as measured by CD19+CD5+ B-cells on flow cytometry. CXCR4 (CD184) is responsible for homing the CLL cells to the protective microenvironment where CXCR4 is subsequently downregulated. Therefore, analysis of the CLL cells by CXCR4 subsets was proposed to represent the subpopulations of CLL cells circulating (CXCR4 high) versus those most recently leaving the protective microenvironment (CXCR4 low-moderate). Antibodies included in the surface phenotype panel included anti-marker (conjugated fluor): CD5 (V450), CD20 (APC-H7), CD19 (FITC), and CD184/CXCR4 (PE) in all tubes; and IgM (PerCP-Cy5.5), CD 38 (PE-Cy7), CD 40 (APC), CD86 (PerCP-Cy5.5), CD80 (PE-Cy7), CD154 (APC), CD23 (PerCP-Cy5.5), CD95 (PE-Cy7), and CD52 (APC). Phospho-protein analysis was performed by phospho-flow cytometry (phosflow) Isolated, fixed B-cells were permeabilized and stained with both extracellular CD5 (V450), CD19 (APC-Cy7), CD20 (PE-Cy7); as well as the following intracellular anti-phospho protein antibodies, p-protein (site): p-syk (pY348), p-p38MAPK(T180/pY182), p-Akt (S473), p-Erk (T202/Y204), p-Mek (S298), pNF-kB (S259), pSTAT3 (S727). All antibodies for flow

and pflow were from BD Biosciences (San Jose, CA) with the exception of CD52 from Invitrogen (Carlsbad, CA). Flow cytometry is performed on a Becton Dickinson LSR II flow cytometer with analysis of results performed with FlowJo software (Tree Star Inc; Ashland, OR). CD52 ELISA Kit/Human CAMPATH-1 antigen (CD52) ELISA was manufactured by CUSABIO (Wuhan, China). Statistical analysis was completed using JMP v. 9-11 by SAS Institute software (Cary, NC).

Results: A total of 15 subjects had correlative samples processed for surface immunophenotyping and pflow during their respective treatment courses amounting to 67 individual timepoint specimens for both surface immunophenotyping panel and the pflow. The gating strategies for defining CXCR4 subpopulations (hi, mod, low) in the analysis, and representation of the changing subpopulations over the treatment course are indicated in Figure 1. Although total WBC decreased over the correlative timepoints, the proportion of the high CXCR4 population increased with time.

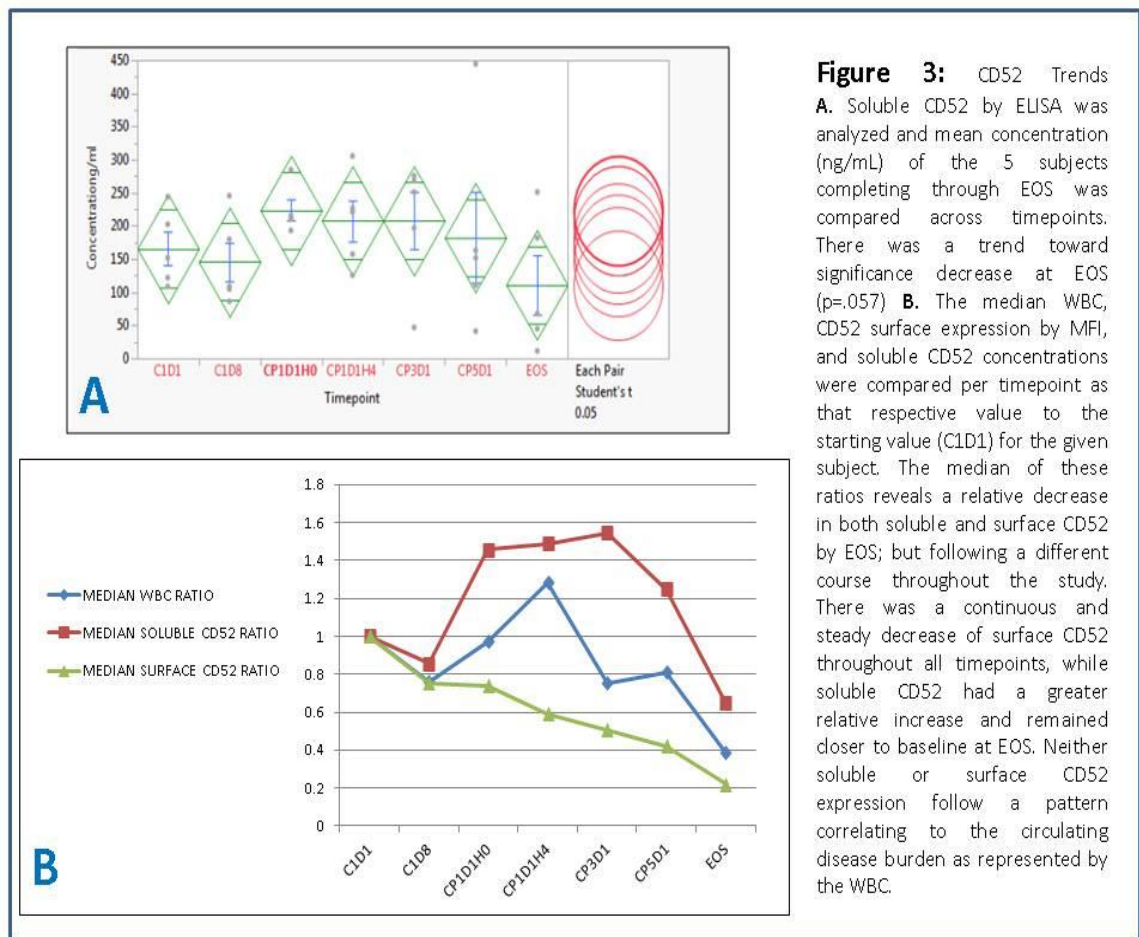
Phenotyping demonstrated CD40, CD95, CD80 and CD86 significantly increased within the CXCR4 high population 4 hours post first plerixafor dose; and for the five subjects completing greater than 4 months of combination treatment with end on study (EOS) samples, CD52 declined significantly. In this same group, phosflow analysis demonstrated a significant increase of p-Erk (T202/Y204) by EOS; these results are displayed in Figure 2.



Given the noted decrease in CD52 surface expression over the treatment course, we performed serum ELISA in the same subject group to assess for variation in soluble CD52. There was a similar trend to decrease in the mean concentration of soluble CD52 through the end of study, though this was not statistically significant ($p=.057$). To examine how the soluble CD52 was changing in comparison to the given subject's starting concentration, the ratio of each timepoint concentration to the respective cycle 1 day 1 (C1D1) concentration was calculated for all 5 subjects. The compiled median soluble CD52 ratio for each timepoint demonstrated a relative increase during lenalidomide monotherapy which remained elevated until four

months of combination (CP5D1) while the comparison surface CD52 ratio was declining from the start. As a method to examine if CD52 was a marker of disease burden of circulating white blood cells (WBC), the ratio of WBC changes was also calculated but followed a different pattern of increase and decrease compared to the CD52. These results are displayed in Figure 3.

Discussion: The longitudinal analysis of surface marker expression and intracellular p-protein changes while subjects were on the clinical trial of the novel combination of lenalidomide and plerixafor provides insight on possible markers or response or resistance. For example, though the total WBC compilation decreased compared to baseline, the CXCR4 high subgroup increased by the end of study. This suggested cells either became resistant to the plerixafor, or cells with lower CXCR4 expression were more susceptible to killing by ongoing treatment (single agent lenalidomide or the combination). Increases of CD40, CD95, CD80 and CD86 on lenalidomide monotherapy within 4 hours of plerixafor addition suggest possible immunomodulatory actions of lenalidomide in circulating CLL cells (CXCR4 high expressing) as these markers are responsible for cell-cell interactions of CLL cells and other microenvironmental cells (predominantly T-cells). However, why this trend did not continue throughout the remainder of the trial is unclear. It is possible that this observation is lost with the confounding variable of the plerixafor addition, or because of the other host related complexities on the treatment.



The changes in CD52 were an interesting finding. In addition to previously published work linking CD52 expression to therapeutic interventions (ie. CAMPATH-

6), high CD52 has also been associated to poorer CLL outcomes. [9] The significant decrease in surface CD52 suggests a possible therapeutic effect given this subject subset did not have disease progression while on treatment. Rather, all that elected to come off study before the 12 cycles of combination did so for treatment related toxicities (predominantly cytopenias). The relative pattern of soluble CD52, surface CD52, and WBC changes over the treatment timecourse did not support that the CD52 findings were predominantly because of the changes in circulating disease burden.

Increased p-Erk by the end of trial may be reflective of an emerging resistance marker despite these patients were not demonstrating overt progression at the time they came off the trial. Previously published studies have shown that CXCL12, released by the microenvironment to overcome spontaneous or drug mediated apoptosis, can induce p-Erk activation. Antibodies to CXCL12 or inhibitors to CXCR4 *in vitro* can block this pro-survival effect. [10] Therefore it is possible that there were microenvironmental adaptive changes that were occurring throughout the treatment course, including a direct resistance to plerixafor's ability to inhibit the CXCR4 receptor.

2.Subtask #2: Examine changes in autocrine and paracrine pro-survival signaling mediated through upregulation of A Proliferation-Inducing Ligand (APRIL) and/or its receptors: APRIL, a TNF family ligand expressed by CLL as a transmembrane protein and a soluble ligand, and secreted by supportive stromal cells in the microenvironment, is a powerful autocrine and paracrine signal for survival [11,12]. Though related TNF family member BAFF can rescue CLL cells from spontaneous and drug induced apoptosis [13], we chose to focus on APRIL as it appears to be the dominant factor in CLL pathogenesis: Increased APRIL, but not BAFF, correlates with adverse prognosis in CLL [14, 15]; and APRIL transgenic mice develop a CD5+ B-LPD with a CLL-like phenotype that can be prevented by treatment of mice with APRIL specific receptor antibodies despite ongoing BAFF signaling. [16, 17] It was hypothesized that induced changes in APRIL or its receptors (TACI and BCMA) during the course of treatment may reveal early development of microenvironmental resistance mechanisms.

Methods: Serum APRIL ELISAs from Invitrogen/Life Technologies (Grand Island, NY) were performed on plasma per the manufacturer. [18-20] Included were all 67 timepoint samples of the 15 subjects with at least one correlative sample; as well as on one normal blood donor. Flow cytometry was performed and analyzed as described for the titer of antibodies for APRIL receptors that included the following anti-marker (conjugated fluor): CD267/TACI-(PE) & CD268/BCMA-(PE) from BioLegend (San Diego, CA), and CD256/APRIL-(APC) from R&D Systems (Minneapolis, MN).

Results: Analysis of soluble APRIL on the 10 subjects who initiated on plerixafor combination treatment is described in Figure 4. The majority (7 subjects) had APRIL serum levels that remained 7ng/mL or less throughout the treatment course. Two subjects had values in the 10-25 ng/mL concentration range; and a single subject had levels greater than 55 ng/mL. Flow cytometry titers of the APRIL surface receptors (APRIL-R, TACI and BCMA) failed to demonstrate a positive and negative population despite testing on several cell types from 3 different subjects with untreated CLL including isolated B-cells, peripheral blood mononuclear cells (PBMC), or whole blood (WB); there also was not a clear positive and negative population when evaluated on the WB of normal control.

Discussion: There were no clear patterns of relative increase or decrease of soluble APRIL levels on the combination of lenalidomide or plerixafor for the subjects on the

trial. The majority of subjects had soluble APRIL at or below reported concentrations for patients with CLL. [15] The subjects that had moderate levels in the 10-25 ng/mL range have no biomarkers that appear to differentiate them from those with very low to undetectable levels. The single subject with the very elevated soluble CD52 interestingly had previously been treated on a study of a PI3K delta isoform inhibitor one month prior to starting this trial. Evidence demonstrates that APRIL can be both a paracrine and autocrine signal for survival thought to be primarily through the PI3K/Akt pathway in both hematological malignancies and solid tumors. [21] It is therefore possible that this subject's resistance to prior PI3Kdelta inhibition was because of an overdrive of this pathway too great to be overcome by the inhibitor, or that the APRIL levels increased as a feedback mechanism to the PI3K pathway inhibition. Interestingly, the phospho analysis of this subject did not indicate high baseline p-Akt as compared to other subjects which may be an indication that the APRIL effects could be signaling down other critical pathways of survival and perhaps why the PI3Kdelta inhibitor alone was insufficient.

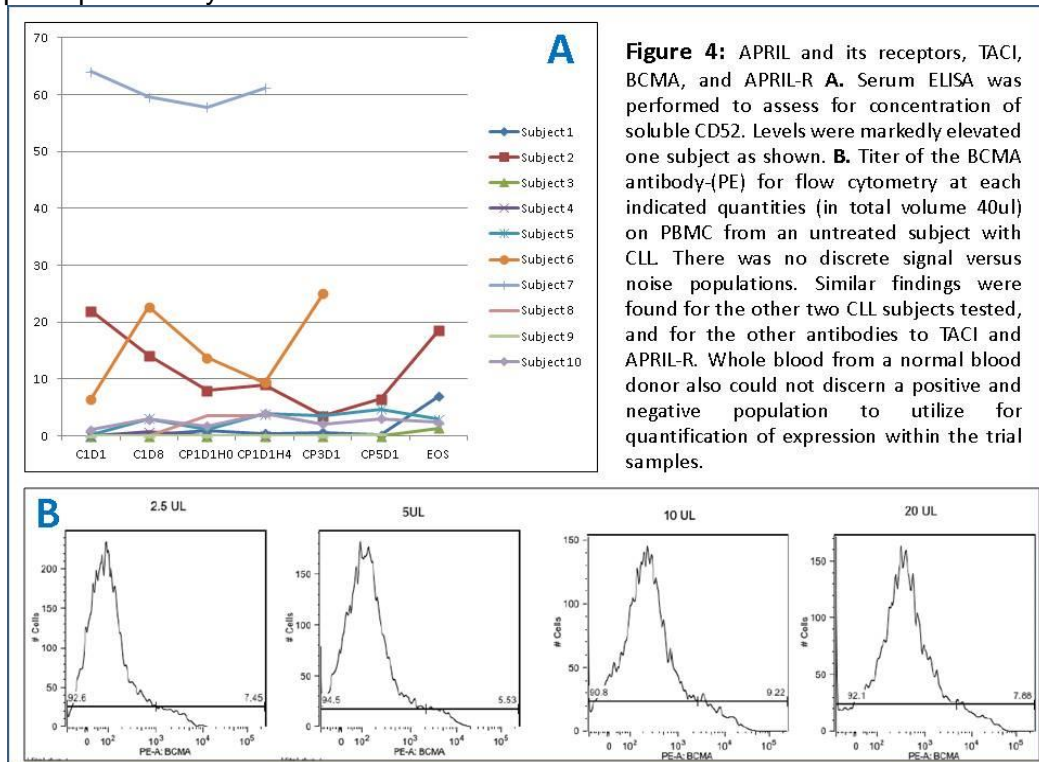


Figure 4: APRIL and its receptors, TACI, BCMA, and APRIL-R. **A.** Serum ELISA was performed to assess for concentration of soluble CD52. Levels were markedly elevated one subject as shown. **B.** Titer of the BCMA antibody-(PE) for flow cytometry at each indicated quantities (in total volume 40ul) on PBMC from an untreated subject with CLL. There was no discrete signal versus noise populations. Similar findings were found for the other two CLL subjects tested, and for the other antibodies to TACI and APRIL-R. Whole blood from a normal blood donor also could not discern a positive and negative population to utilize for quantification of expression within the trial samples.

The planned accompanying analysis to soluble APRIL levels was quantifying APRIL receptors by flow cytometry (BCMA, TACI, APRIL). Unfortunately despite testing the antibodies on various cell populations of 3 different subjects with CLL, and on one normal subject, a clear positive and negative (or signal to noise) could not be determined. Therefore study samples were not analyzed for surface expression by flow since there was no clear indication these flow antibodies could discern surface markers on the correlatives. Moving forward we have identified several alternative options for analysis of these surface markers/APRIL receptors: (1) obtain antibodies from other companies (though limited in the variety of fluor available, restricting the ability to combine markers within the same flow tube and thereby necessitating more cells), (2) evaluate the soluble TACI, BCMA, and BAFF by ELISA. [22] Interestingly, soluble BCMA has been reported to bind APRIL and decrease its effect. Looking for inverse correlation of the soluble BCMA in high APRIL, or co-incubation with PI3K

pathway inhibitor and soluble BCMA in previously untreated cells, could provide more insight in this area. [23]

3. Subtask #3: Evaluate the disruption of drug induced apoptotic mechanisms: Though preclinical studies of the novel small molecule inhibitors demonstrate that they also kill through apoptosis as recognized for the traditional cytotoxic chemo(immune)therapies [24-25], the specifics of the molecular mechanisms of apoptotic block leading to acquired drug resistance was not well established.

1. Subtask 3A: Changes in expression profile of pro and anti-apoptotic proteins. Quantitative Drug resistance to apoptosis can ensue as malignant cells are equipped to evolve blocks in apoptosis through upregulation of anti-apoptotic proteins (Bcl-2, Mcl-1), and/or suppression of pro-apoptotic death proteins (ie BAX, BAK) [26]. The hypothesis of the evaluation was that expression differences of the respective proteins may vary over the course of the treatment for the correlative samples, on between different CLL patient cells *in vitro* when exposed to the different targeted inhibitors to BTK, PI3K or MEK. Reverse-Transcriptase PCR (RT-PCR) was planned for quantifying the expression of an apoptotic panel including anti-apoptotic Bcl-2, Mcl-1; and pro-apoptotic BAX, BAK, and BIM. However, ongoing evidence particularly in the field of the novel BCL-2 inhibitors suggests that responsiveness or resistance to inhibitors of apoptosis is not directly correlated to expression levels of these proteins. For example, key aspects of the BH3/mitochondrial priming is also needed for assessment of the readiness of the cell for apoptotic death. [27, 28]

Based on this important current research, we have updated our research plan to include the optimization of a flow based system of BH3 profiling/mitochondrial profiling in CLL to coincide with selected expression profile. New flow based quantitative methods by Affimetrix (ebioscience, San Diego, VA) allow measurement of up to 3 different mRNA transcript levels per tube, thereby allowing up to discern on the single cell level how these anti- and pro-apoptotic protein expression ratios compare. [29, 30] In addition, we recognized that expression levels of the pro and anti-apoptotic proteins would need to be understood in the context of other critical components of the apoptotic machinery such as caspases, and therefore we embarked on the caspase experiments per subtask 3B.

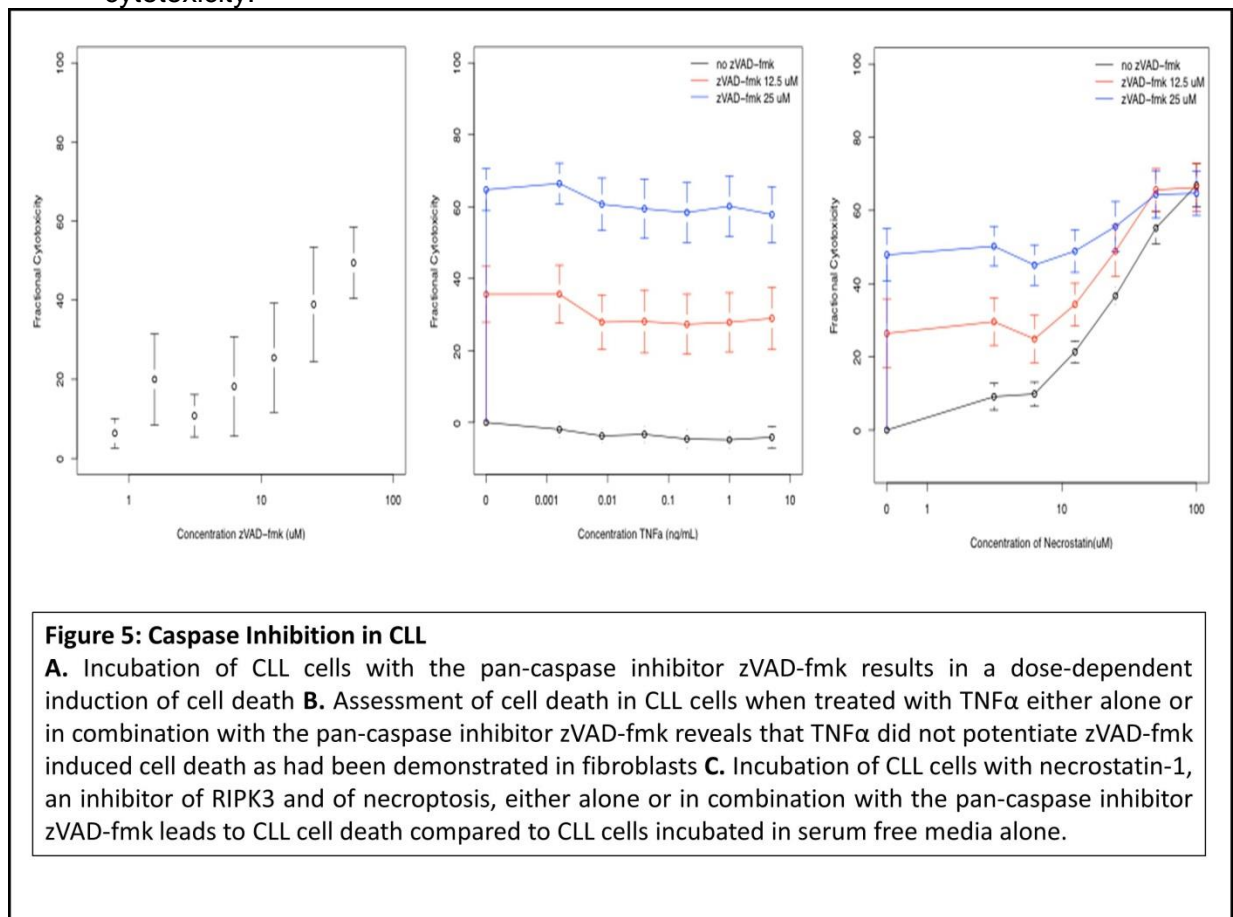
2. Subtask 3B: Alterations in caspases as part of the apoptotic cascade.

Caspases are enzymes in the mitochondria that are responsible for cleavage of proteins as part of the programmed cell death cascade. We hypothesized that *in vitro* inhibition of caspases will protect CLL cells from drug-induced apoptosis. We also hypothesized that inhibition of caspases will have a negligible effect of CLL cells *in vitro*.

Methods: We performed *in vitro* evaluation of cell death in purified CLL cells.

The CLL cells were purified from blood collected from CLL patients collected and purified as previously described. We then tested the cytotoxic effect of the pan-caspase inhibitor zVAD-fmk (Promega, Durham, NC) in serial dilutions with 2.5×10^5 CLL cells after three days of incubation at 37°C and 5% CO₂, using the MTS assay (CellTiter 96 Aqueous One solution, Promega). We also tested necrostatin-1 (gift of the Pisetsky laboratory, Duke University, Durham, NC), an inhibitor of an alternate mechanism of cell death called necroptosis, in serial dilutions with CLL cells using the MTS reagent. Lastly, we tested serial dilutions of zVAD-fmk in combination with necrostatin-1 or in combination with TNF- α (Promega) again with the MTS reagent.

Results: Unexpectedly, when CLL cells are incubated with the pan-caspase inhibitor zVAD-fmk, there is a dose-dependent induction of cell death (Figure 5A, n = 5). Compared to CLL cells incubated with serum free media alone, 25 μ M of zVAD-fmk induced cytotoxicity in approximately 50% of CLL cells. Necroptosis is a non-apoptotic cell death mechanism. It was initially defined in fibroblasts as cell death that is induced by caspase inhibitors and is potentiated by TNF α . However, in CLL, TNF α has been shown to have a mild protective effect in vitro. Since CLL cells were dying with zVAD-fmk, we hypothesized that zVAD-fmk might be inducing cell death via necroptosis. Thus, we assessed cell death in CLL cells when treated with TNF α either alone or in combination with the pan-caspase inhibitor zVAD-fmk (Figure 5B, n = 5). Contrary to what was demonstrated in fibroblasts, TNF α did not potentiate zVAD-fmk induced cell death. In fact, it had a mild protective effect. To further characterize if zVAD-fmk induced necroptosis, we incubated CLL cells with necrostatin-1, an inhibitor of RIPK3 and of necroptosis, either alone or in combination with the pan-caspase inhibitor zVAD-fmk (Figure 5C, n = 7). Again, unexpectedly, necrostatin-1 itself induced CLL cell death compared to CLL cells incubated in serum free media alone. While we hypothesized that the addition of necrostatin-1 to zVAD-fmk in CLL cells would abrogate cell death, there was potentiation of cytotoxicity when the two compounds were combined, up to a maximum of approximately 65% cytotoxicity.



Discussion and Future Experiments: This series of experiments is meant to set the stage for further evaluation of alterations of apoptotic proteins as a

mechanism of drug resistance as described. In order to understand the extent to which apoptotic proteins affect the response to targeted small molecule inhibitors, we began by evaluating the effect of altering the apoptotic machinery (specifically caspases) on CLL cells cultured *in vitro*. To our surprise, blocking apoptosis by inhibition of caspases using zVAD-fmk induced CLL cell death, rather than protecting or having negligible effect. It does not appear that the mechanism by which zVAD-fmk induces CLL cytotoxicity is necroptosis, based on our experiments with TNF α and necrostatin-1. These results may have importance as new kinase inhibitors are incorporated in the therapeutic armamentarium for CLL, or as BCL2 inhibitors (i.e. apoptosis promoters) move forward in clinical trials. [31, 32]

We are currently confirming our results using an alternate cell death assay, specifically flow cytometry for Annexin V expression and 7AAD staining. We hypothesize that incubation with zVAD-fmk or necrostatin-1 will cause cells to die (7AAD positive) but not undergo apoptosis (Annexin V negative). If this is the case, it would confirm our results with the MTS assay. If it is not the case, this would suggest that zVAD-fmk or necrostatin-1 may affect mitochondrial function or respiration, since MTS measures mitochondrial function as a surrogate for cell viability. We also plan to test alternate caspase inhibitors (such as the specific caspase 3 inhibitor, zDEVD-fmk or specific caspase 8 inhibitor, zLEHD-fmk) to confirm results and assess the extent to which initiating or effector caspases produce similar effects. If the results are confirmed, we plan to assess whether autophagy is a mechanism of cell death induced by the apoptosis and necroptosis inhibitors in CLL cells.

4. Summary of TASK 2 Continuing Experiments during 2014-2016 Award Period:

- Complete soluble CD52 ELISA for the remaining trial correlates of subjects remaining on study as a minimum through the start of plerixafor. The goal is to increase the sample size to look for early changes on lenalidomide monotherapy.
- Assess for soluble TACI and BCMA (function as surface receptors for APRIL) in the clinical trial correlate samples by ELISA. A published assay is available from R&D Systems as described.
- Test whether cytotoxicity is enhanced when PI3K inhibitors are co-incubated with soluble BCMA that could block APRIL.
- Determine the ratio of pro- and anti-apoptotic protein expression in the clinical trial correlate samples by mRNA levels utilizing quantitative RNA flow cytometry. This method has been established by the described kits available through affymetrix ebioscience. Also optimize a system for assessing BH3 priming/apoptosis readiness.
- Confirm the caspase experiments as described utilizing the Annexin V expression and 7AAD staining to assess for death but not apoptosis; and by testing alternative caspase inhibitors such as the specific caspase 3 inhibitor zDEVD-fmk, or specific caspase 8 inhibitor zLEHD-fmk.
- Complete subtasks 1-3 utilizing the drugs that were selected for *in vitro* study on primary isolated B-CLL cells including the inhibitors critical intracellular signaling pathways in B-LPD: inhibitors of Phosphoinositide 3-kinase inhibitor (PI3K), Bruton's Tyrosine Kinase (BTK), and MEK1/2. Drugs to be tested are the commercially available idelalisib and ibrutinib (SelleckChem, Houston, TX), GSK212/trametinib (Chemietek, Indianapolis, IN) along with the controls fludarabine monophosphate and bendamustine (SelleckChem, Houston, TX). We also plan to explore the role of an alternative PI3K-delta inhibitor TGR-1202 and a dual PI3K δ/γ inhibitor, RP6530, after promising *in vitro* work partially contributed by

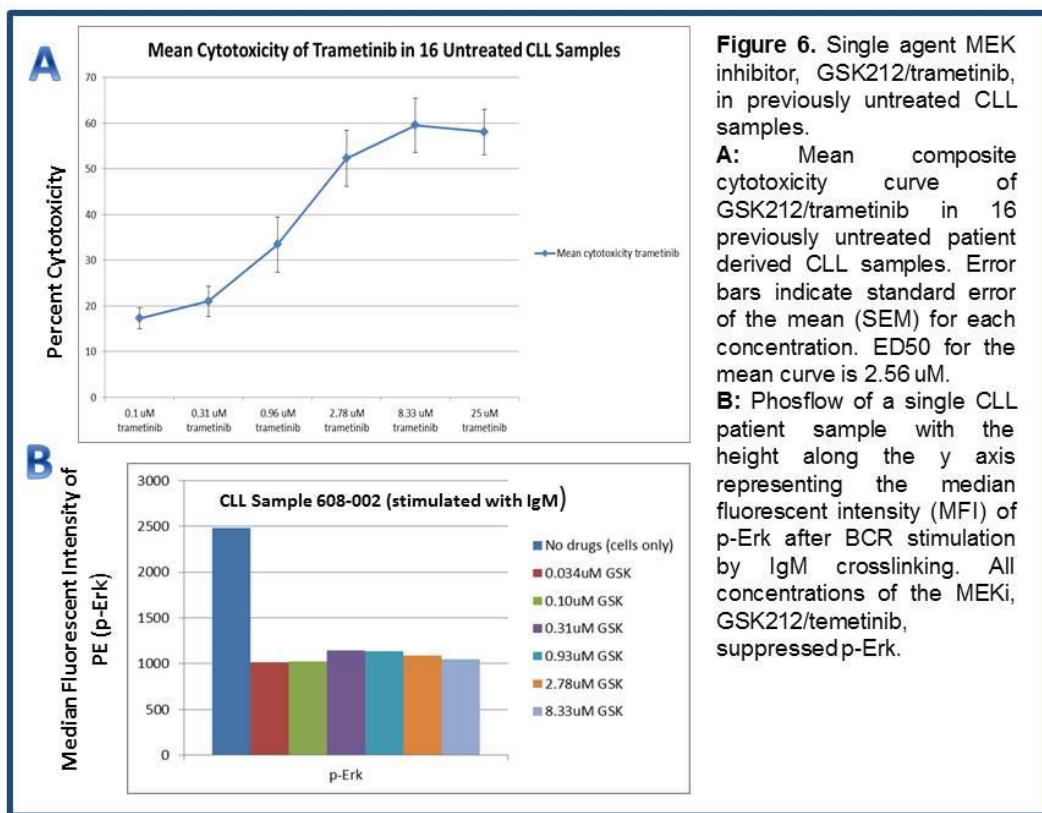
the Lanasa Lab (previous award mentor) and Weinberg Lab (collaborator). [33-34] The experiments will all these drugs will explore the ability of each inhibitor to block extracellular activation of signaling pathways (BCR, CXCR4, and CD40L) by evaluating downstream p-proteins (p-syk, p-Erk, p-MEK, p-Akt and p-NF-kappaB) using pflow. It will also include additional experiments similarly planned for the trial correlates wherein the CLL cells are exposed to the inhibitors *in vitro* and then evaluated for soluble TACI and BCMA by ELISAs; and of the apoptotic machinery (pro/anti-apoptotic protein mRNA by flow; BH3 priming).

C. TASK 3: Define the role of the microenvironment in drug resistance to the targeted small molecule inhibitors

1. **Subtask #1: Identify pathways activated to induce drug resistance by the microenvironment:** Defining the relative contribution of the established pro-survival and anti-apoptotic pathways induced by the microenvironment, along with identification of additional critical pathways in microenvironmental derived drug resistance, is needed. We hypothesized that global gene expression profiling of samples exposed to a concentration of drug established to fall in the therapeutic window (between ED50 dose and the dose which leads to 50% reduction in target protein phosphorylation), compared to the same experiment with cells cultured in a stromal microenvironment can quantify the contribution of established important pathways as well as identify novel microenvironmental pathways involved in drug resistance. Drugs to be tested are the same as identified in TASK 2: commercially available idelalisib, ibrutinib, GSK212/trametinib; and alternative PI3K inhibitors TGR1202 (delta isoform) and RP-6530 (delta/gamma isoform), along with the controls fludarabine monophosphate and bendamustine,

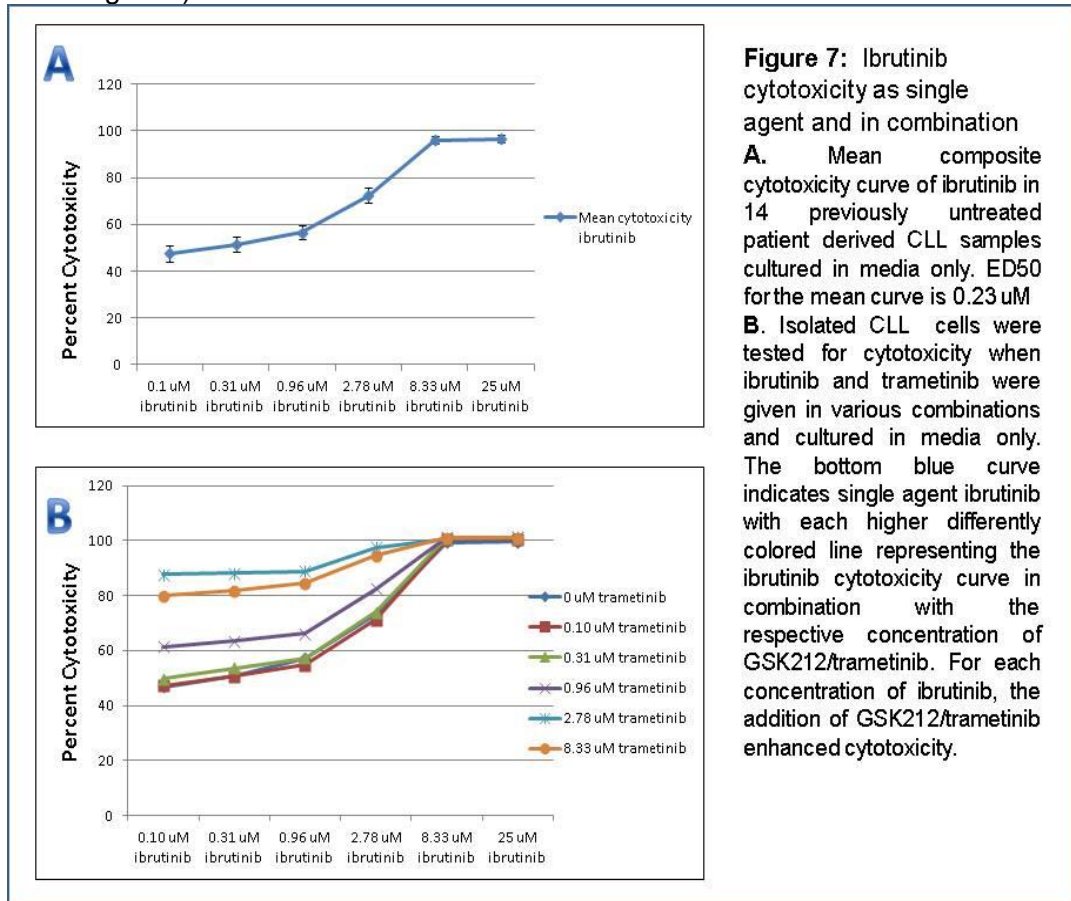
Methods-media only culture: The CLL cells were purified from blood as previously described. In this cytotoxicity assay, isolated CLL cells were incubated in triplicate in a 96-well tissue culture plate (Costar, Corning, NY) with serial dilutions of the inhibitor (based on published data for each respective inhibitor) in Hybridoma serum free media (SFM) (GIBCO/Invitrogen, Carlsbad, CA) at 37°C. The percentage of viable CLL cells following culture for 3 days in Hybridoma serum free media was determined using the tetrazolium compound: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) and the CellTiter 96® Aqueous One Solution Cell Proliferation Assay from Promega (Madison, WI). From this we sought to determine the ED50 for the inhibitor for media only culture. Concurrently, a separate phosflow assay was performed in purified CLL cells to evaluate the downstream target effect of the drug and identify the dose producing a 50% reduction in phosphorylation. First, 0.50×10^6 patient derived cells/well were added to deep well plates in duplicate containing serial dilutions of the inhibitor ranging from 0 (cells only) to a dose selected based on published data for the respective inhibitor. Cells were incubated with drug at 37C for one hour. Next, soluble IgM was added to one plate and the stimulation condition continued for 10 minutes before phosflow fix/lyse buffer (BD) was added and plates were stored at -80C. Isolated, fixed CLL cells were permeabilized and stained with both extracellular (anti-CD19) as well as the intracellular anti-phospho protein pErk-PE (BD, San Jose CA). Flow cytometry was performed on a Becton Dickinson ARIA flow cytometer and analysis was performed with FlowJo software (Tree Star Inc; Ashland, OR). Phosphorylated protein was quantified according to the median fluorescent intensity (MFI) on analysis. Figure 6 demonstrates these experiments testing GSK212/trametinib's cytotoxicity and downstream effect on p-Erk by phosflow

quantification.



Methods-stromal co-culture: The human stromal HS-5 cell line [35] was obtained from the Duke Cell Culture Facility and resuspended in HS-5 media (DMEM with high glucose and non-heated inactivated FBS) according to the American Type Culture Collection (ATCC) product information sheet CRL-11882. Cells were cultured until the recommended confluence was reached with media changed every 2-3 days per standard culture protocol. Twenty four hours prior to a planned co-culture experiment the stromal cells are added to wells of a 96-well tissue culture plate to assure adherence. The CLL cells were isolated as described, and are resuspended at a concentration of 5×10^6 cells/mL. The inhibitor serial dilutions are prepared using serum free media. Fludarabine and bendamustine served as the controls; fludarabine was diluted 1:2 from a starting 50uM to 3.125uM, and bendamustine was diluted 1:3 from 167uM to 2.05uM. Drugs were added both to a plate containing serum free media and one with the stromal co-cultures, and the same subject isolated CLL cells added to both at 0.5×10^6 cells in 100uL. Both plates per subject were incubated for 72 hours at 37C and 5% CO2. Initially the MTS assay was utilized to assess cytotoxicity of cells tryponized cells but the stromal cells interfered with the ability of this assay. Therefore we subsequently optimized the flow based assay for caspase-3 and 7-AAD to assess for death and apoptosis, and also determined that removal of viable cells was equivalent for vigorous pipetting methods compared to tryponized. As the later could introduce changes in the biology of the cells, a vigorous pipetting method was used for removal of the CLL cells from the stromal co-culture moving forward. Cells were stained for 7-AAD-(PE-Cy5) (Life Technologies,), active caspase 3-(PE) (BD Biosciences), and CD45-(APC) (BD biosciences); CD45 was used to gate out the stromal cells as this could not be determined by forward and side scatter

alone properties. Flow and analysis was performed per the lab standard. After gating strategies the percentage of death was determined as 1- (caspase 3 negative and 7-AAD negative).



Results: The ED50 for GSK212/trametinib by MTS was established at 2.56 uM for media only culture of the isolated CLL cells averaged for 16 subjects; the dose producing 50% dose reduction of the downstream p-Erk was not obtained as per Figure 6. The ED50 of ibrutinib using the mean of 14 subject MTS assays was 0.229uM per Figure 7; the dose producing 50% dose reduction of the p-BTK has not yet been determined given the p-flow p-BTK antibody could not detect differences in control situations (data not shown). Five subjects were subsequently studied to assess the ED50 of selected inhibitors and standard chemotherapeutic drugs as single agents and combination on media only; the ED50s from these experiments included ibrutinib: 57 uM, fludarabine: 14.8 uM, bendamustine 380.3 uM, and .36 uM. These are graphically depicted in Figure 8.

The stromal co-culture experiments, considering the single agent and combinatory studies, to date have evaluated 8 subjects for fludarabine, 5 subjects with bendamustine, 3 with ibrutinib, 9 with PI3K inhibitors (6 with TGR1202 and 3 with RP6530). Provided in Appendix are example flow plots and charts with cytotoxicity data; the ED50 has not been adequately assessed for the inhibitors of interest in this setting. No phosflow experiments for intracellular proteins have been completed to determine the dose of inhibitor that produces the 50% reduction in the target protein.

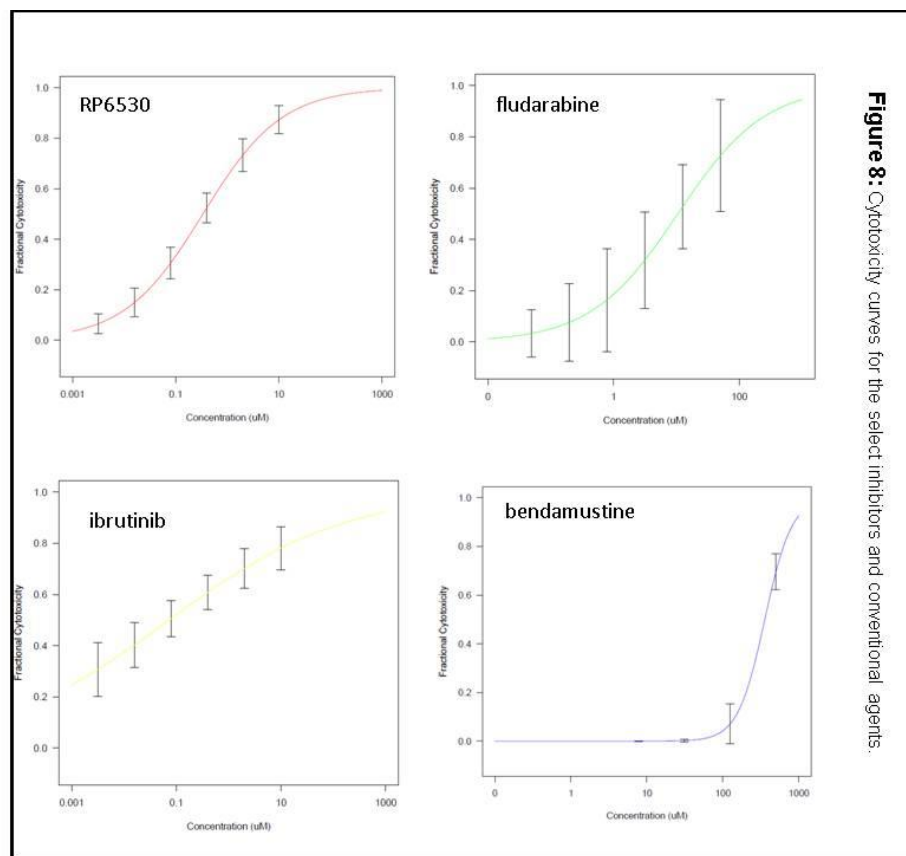


Figure 8: Cytotoxicity curves for the select inhibitors and conventional agents.

Discussion: These experiments found lower ED50s of the selected inhibitors including ibrutinib which has been well characterized. This may be due to selection of CLL cells from previously untreated subjects in our assays. Moving forward, we will consider specific analysis of previously treated patients and those with high risk genetic features (del17p or del11q) to gain a more comprehensive and representative assessment.

The initial co-culture results were confounded by limitations in the assays used in data analysis: The MTS assay did not provide accurate results because of interference by the stromal cells. When moving to a flow based assay (7-AAD to stain for dead cells, and caspase-3 to stain for cells undergoing apoptosis; designating the double negatives as the proportion of living cells); it remained difficult to exclude any contaminating stromal cells. Therefore CD45 was added to the flow panel, with positive CD45 population enabling selection of the CLL cells with exclusion of the stromal cells. The stromal cells protect from drug mediated apoptosis and death, requiring adjustment of concentrations utilized. Studying drugs in combination provides an internal control of both drugs as single agents within the same subject, and also provides insight on two interesting observations were noted. First, the fludarabine co-culture treated CLL cells had enhanced cell killing, when one would have expected the stromal cells to be protective. Follow up experiments of fludarabine on the stromal cells indicated that the fludarabine even at low concentrations was directly cytotoxic to the stromal cells thereby enhancing the death of any CLL cells added to these wells. Secondly, in combination studies of ibrutinib and the dual PI3K gamma/delta inhibitor RP-6530, the ibrutinib appeared more cytotoxic as a single agent but the stromal cells provided greater protection than for

RP-6530. The RP-6530 may have different mechanisms to explain overcoming the protective properties of the stromal cells. Additional experiments will examine these trends, including use of alternative stromal systems to observe if the properties are similar for the same drugs.

2. Summary TASK 3 Continuing Experiments during 2014-2016 Award Period:

- Establish the ED50s and the dose of drug producing 50% reduction in target protein phosphorylation for the remaining inhibitors and conditions (co-culture versus media alone) not defined above.
- Perform gene expression arrays for a given previously untreated CLL subject exposed separately for each inhibitor class with the drug concentration falling within the therapeutic window; samples are paired for CLL cells cultured on a stromal co-culture system versus media alone. We plan to compare the paired samples for contribution of differentially induced drug resistance pathways by the microenvironment.
- Optimize an additional co-culture system and compare laboratory and biological differences. The StromaNKTert from RIKEN cell laboratories (Japan) has been well established in other laboratories and in particular to the work with apoptotic inhibitors and BH3 priming. [36]
- Observe quantitative biological differences between the co-culture of the cells at a given concentration on at least 2 different co-culture systems.

IV. MAJOR RESEARCH ACCOMPLISHMENTS: Nothing to report

V. CONCLUSION:

The aims of this project were to explore mechanisms of resistance specific to the microenvironment, and to the novel small molecule inhibitors (including those targeting BTK, MEK, and PI3K) based on the drug target and interactions with supportive cells of the microenvironment. The data derived from subtasks of TASK 2 and 3 provided valuable insight to potential mechanisms of resistance as detailed in the discussion of each individual subtask.

In summary, we successfully completed studies on the individual 67 timepoint correlative samples from 15 subjects enrolled on the clinical trial of the novel microenvironmentally targeting combination of lenalidomide and plerixafor. For the longitudinal analysis of surface marker expression and intracellular p-protein changes on the trial, we found that though the total WBC compilation decreased compared to baseline, the CXCR4 high subgroup increased by the end of study. This suggests cells became resistant to the plerixafor or chemotherapy in general, by increasing CXCR4 expression over time. Increased CXCR4 expression would enable to cells to migrate to the protective niches of the bone marrow or lymph nodes, shielded from the cytotoxic effect of treatment. Increases of CD40, CD95, CD80 and CD86 on lenalidomide monotherapy within 4 hours of plerixafor addition suggest possible immunomodulatory actions of lenalidomide in circulating CLL cells (CXCR4 high expressing) as these markers are responsible for cell-cell interactions of CLL cells and other microenvironmental cells (predominantly T-cells). This did not continue throughout the remainder of the trial possibly because of the confounding variable of long term plerixafor addition. The changes in CD52 were an interesting finding given published data that high CD52 has also been associated to poorer CLL outcomes. In the correlates we tested of subjects remaining on trial, the significant decrease in surface CD52 suggests a possible therapeutic effect given this subject subset did not have disease progression while

on treatment. The relative pattern of soluble CD52, surface CD52, and WBC changes over the treatment timecourse did not support that the CD52 findings were predominantly only a marker of the changes in circulating disease burden. Finally, the increased p-Erk by the end of trial may be reflective of an emerging resistance marker despite these patients were not demonstrating overt progression at the time they came off the trial. Previously published studies have shown that CXCL12, released by the microenvironment to overcome spontaneous or drug mediated apoptosis, can induce p-Erk activation. This would fit with the observed increased expression of CXCR4, the receptor for CXCL12 despite the stable WBC in these subjects. That is, this could be a potential mechanism at a cellular level of developing resistance that subsequently may evolve into disease progression if they had remained on study for an additional timeperiod.

Examination of changes in APRIL and its receptors in the correlatives revealed that the majority of subjects had soluble APRIL at or below reported concentrations for patients with CLL. However, the single subject with the very elevated soluble APRIL interestingly had previously been treated on a study of a PI3K delta isoform inhibitor. As published evidence demonstrates that APRIL primarily functions through the PI3K/Akt pathway, the elevated APRIL may have been a feedback mechanism for the therapeutic pathway blockade. Surface receptors for APRIL, TACI and BCMA, were not able to be quantified due to the nonspecific nature of binding to various cell subpopulations in CLL subjects and normal control. Future experiments to block APRIL through soluble BCMA, concurrent with the PI3K pathway inhibitor, may provide additional insight. In addition, quantifying the soluble APRIL receptors BMCA and TACI could inversely suggest mechanisms of resistance if the soluble BCMA binding can prevent APRIL effects.

Further exploration of disruption in the normal apoptotic machinery as a mechanism of resistance included foundation experiments looking at inhibition of caspases using zVAD-fmk. This inhibition induced CLL cell death, rather than protecting or having negligible effect. It does not appear that the mechanism by which zVAD-fmk induces CLL cytotoxicity is necroptosis, based on our experiments with TNF α and necrostatin-1. These results may have importance as new kinase inhibitors are incorporated in the therapeutic armamentarium for CLL, or as BCL2 inhibitors (i.e. apoptosis promoters) move forward in clinical trials. Future experiments will aim to support these results, as well as look at other recently confirmed vital components of the apoptotic machinery including BH3/mitochondrial priming, and ratios of the pro to anti-apoptotic proteins. Novel flow based assays are planned that can concurrently quantify multiple mRNA transcripts on a cellular cell enabling correct ratio comparisons.

During this report period we have successfully obtained and optimized the first stromal co-culture system for our lab, along with a flow based cytotoxicity/apoptosis assay for drug/inhibitor testing. This has been the platform for evaluation of the protective features of the stroma microenvironment when cells are treated with the inhibitors of interest. Determination of the ED50 and 50% target protein lowering dose of each inhibitor will enable performance of gene expression arrays looking at differences with cells treated within this therapeutic window that are cultured in media versus the stroma co-cultures. Recognition of these critical pathways can highlight the complexity or necessary protective components for the differing stromal systems available, and potentially provide insight on identifying the most appropriate system for the indolent B-cell malignancies.

In conclusion, during the past year we have gained valuable insight into possible mechanisms of resistance to the targeted inhibitors, and supports stimulating future experiments during the remaining award period. This experience has provided exciting opportunities in laboratory research with potential clinical applications; as well as the invaluable protected time for hands on mentoring, attendance and submission of abstracts to pivotal societal (ASH) meetings, and application to additional funding and career

development programs. Together this supports a foundation imperative to achieving future success as an academic research physician in hematological malignancies.

VI. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. ABSTRACTS:

1. **Brander DM**, Allgood SD, Simms TM, Weinberg JB, and Lanasa MC: Laboratory Correlates and a Phase I Clinical Trial Of Lenalidomide In Combination With Plerixafor In Patients With Previously Treated Chronic Lymphocytic Leukemia. [Abstract, Published Online]. American Society of Hematology Annual Meeting. December 9-12, 2013.
2. **Brander DM**, Allgood SD, Rizzieri D, Simms TM, Weinberg JB, and Lanasa MC. Final Clinical Results with Laboratory Correlates in the Phase I Trial of Lenalidomide Plus Plerixafor in Previously Treated Chronic Lymphocytic Leukemia (CLL). [Abstract, Submitted]. American Society of Hematology Annual Meeting. December 2014.

b. PRESENTATIONS:

1. Coincidence or Causes: CLL and Pleural Effusions. Duke CME Activity, Hematological Malignancies Grand Rounds. May 16th, 2014.
2. Ibrutinib for Frontline CLL Therapy? The Duke Debates in Hematological Malignancies, regional Duke CME activity. The Mills House, Charleston, SC, March 14-16th, 2014.
3. Putting the "I" in Frontline: The future role of ibrutinib and idelalisib as first therapy for patients with CLL with special considerations on toxicities and drug resistance. Duke CME Activity, Hematological Malignancies Grand Rounds. August 22nd, 2014.

VII. INVENTIONS, PATENTS AND LICENSES: Nothing to report

VIII. REPORTABLE OUTCOMES: Nothing to report

IX. OTHER ACHIEVEMENTS:

A. Funding Applications

1. Lymphoma Research Foundation Clinical Research Mentoring Program: provides \$10,000 for career development activities. *Submitted 3 September 2014.*
2. The National Institutes of Health Loan Repayment Program (NIH LRP) renewal application. *In Process.*

X. FELLOWSHIP AWARD CAREER DEVELOPMENT ACTIVITIES: As stated above, the CDMRP Visionary Postdoctoral Fellowship Award has provided a tremendous support mechanism for successful advancement in my career development path. Below highlights some of these career development opportunities:

A. Protected time with diverse mentors in the field (see Appendix for CVs)

1. Project Mentor Daphne Friedman: The award has allowed us to meet individually at minimum 2 hours per week with additional time in Weinberg lab meetings. During the individual time with Dr. Friedman we review data,

troubleshoot experimental difficulties, and plan future experiments that incorporate our data with novel data in the field.

2. J. Brice Weinberg: Dr. Weinberg has over 40 years of experience in medical research, and established the IRB approved collection mechanism that provides the vital patient derived CLL cells. Importantly he has mentored over 50 individuals; progressing to pursuing MDs or PhDs (16), and to positions in academic institutions and medical school settings (26).
 3. Sandeep Dave: Dr. Dave has become a well-recognized international expert in the genomics of lymphomas; and also has been a successful mentor to early career physician scientists. Dr. Dave has mentored me in the important nuances of array analysis and applying the data to future experimental questions.
 4. David Rizzieri: Dr. Rizzieri leads the clinical trials in our division with well recognized leadership in early phase trials with correlatives.
- B.** Broadened my laboratory research repertoire in techniques I have not performed prior, and in novel experimental components that have the potential to improve the depth and quality of the proposed research:
1. Stromal co-culture systems
 2. ELISAs
 3. Quantitative mRNA by flow cytometry
 4. Western Blots
 5. Array analysis
- C.** Enabled attendance at pivotal societal meetings:
1. ASH 2013 (abstract submitted, published online)
 2. ASH 2014 (abstract submitted)

XI. REFERENCES:

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XII. APPENDICES:

- A. Phase I Clinical Trial Timeline of Events
- B. Stromal Co-culture systems representative flow analysis and data
- C. CV-Danielle Brander, MD (PI)
- D. CV of Mentors
 - 1. Daphne Friedman, project/proposal early career mentor
 - 2. J. Brice Weinberg, collaborator and mentor in malignant B-cell laboratory research
 - 3. David Rizzieri, Clinical research mentor including design of clinical trials with laboratory correlatives
 - 4. Sandeep Dave, Mentor in Genomics and Translational Research

APPENDIX A: Clinical Trial Correlates

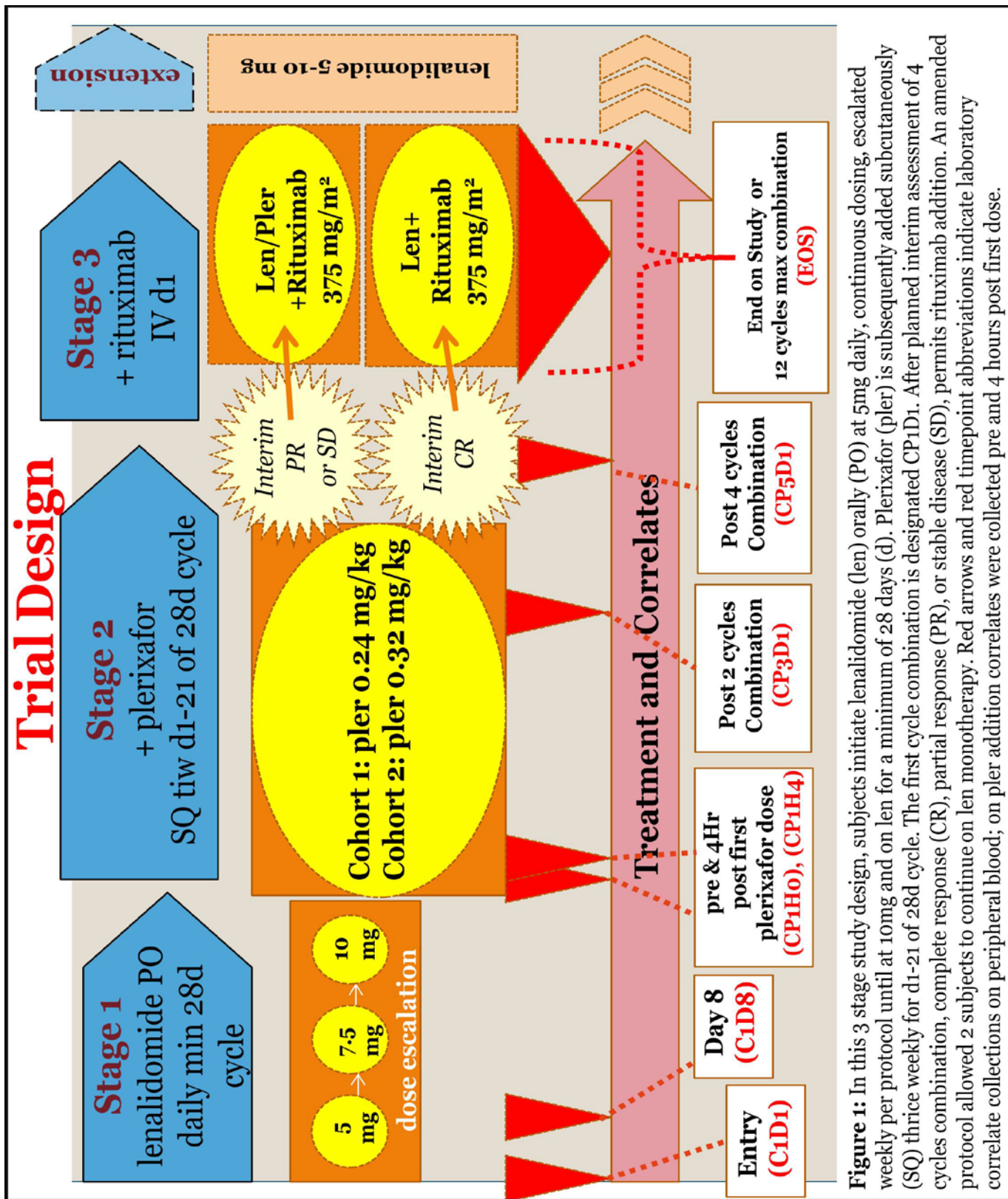


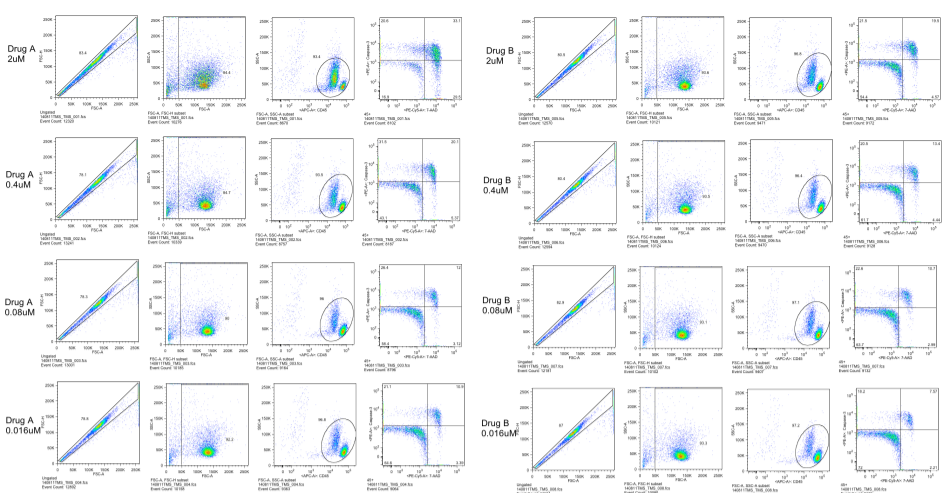
Figure 1: In this 3 stage study design, subjects initiate lenalidomide (len) orally (PO) at 5mg daily, continuous dosing, escalated weekly per protocol until at 10mg and on len for a minimum of 28 days (d). Plerixafor (pler) is subsequently added subcutaneously (SQ) thrice weekly for d1-21 of 28d cycle. The first cycle combination is designated CP1D1. After planned interim assessment of 4 cycles combination, complete response (CR), partial response (PR), or stable disease (SD), permits rituximab addition. An amended protocol allowed 2 subjects to continue on len monotherapy. Red arrows and red timepoint abbreviations indicate laboratory correlate collections on peripheral blood; on pler addition correlates were collected pre and 4 hours post first dose.

APPENDIX B: Stromal Co-culture systems:

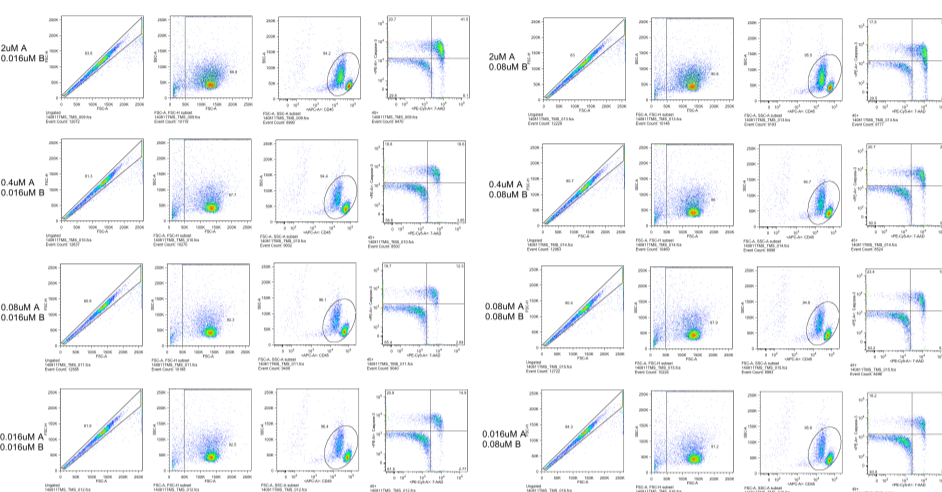
- 1. Combination study of ibrutinib (Drug A) and RP-6530 (Drug B)**
 - a. CLL cells only: cultured in media**
 - b. Stromal cells: co-culture of CLL cells on the stromal system and HS-5 media**
- 2. Combination study of ibrutinib (Drug A) and trametinib (Drug B)**
 - a. CLL cells only: cultured in media**
 - b. Stromal cells: co-culture of CLL cells on the stromal system and HS-5 media**

Ibrutinib and RP6530 as single agents and in combination treatment of primary CLL cells cultured in media (CLL only) or on stromal cells (Stromal cells) using a flow cytometry assay for drug induced apoptosis and cell death

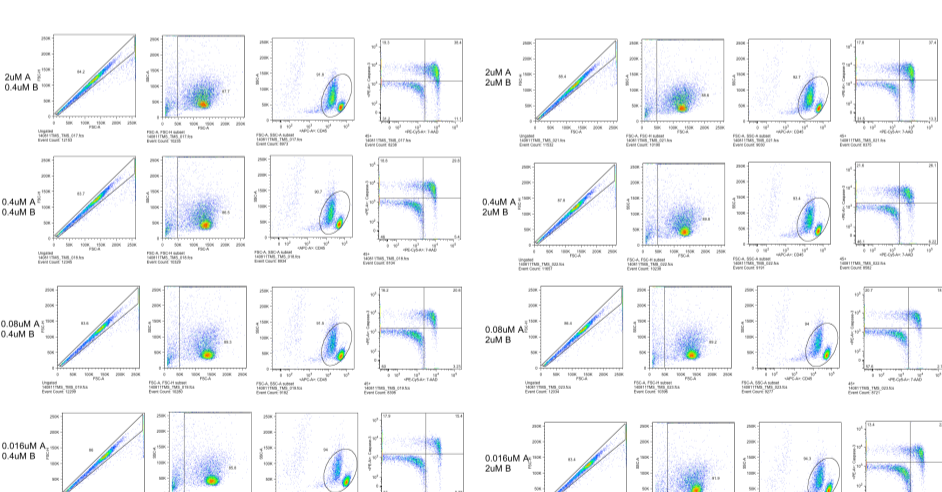
171-017 CLL Only



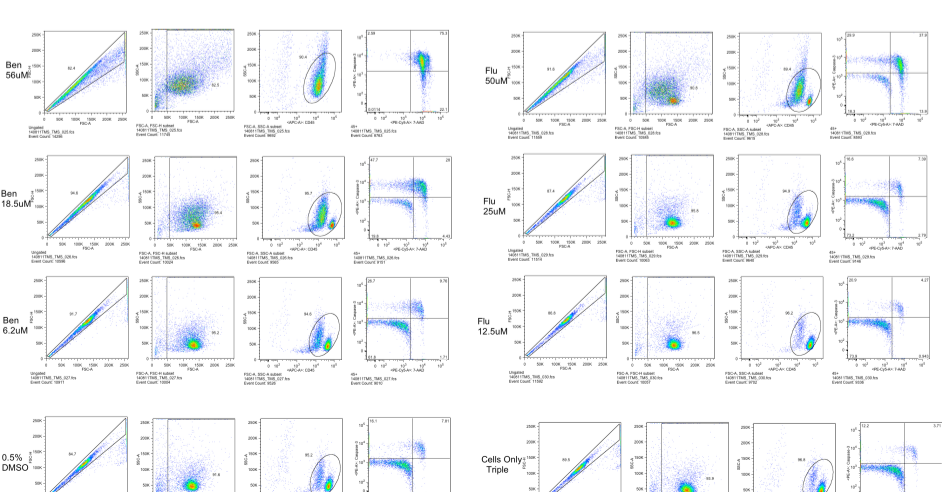
171-017 CLL Only, cont.



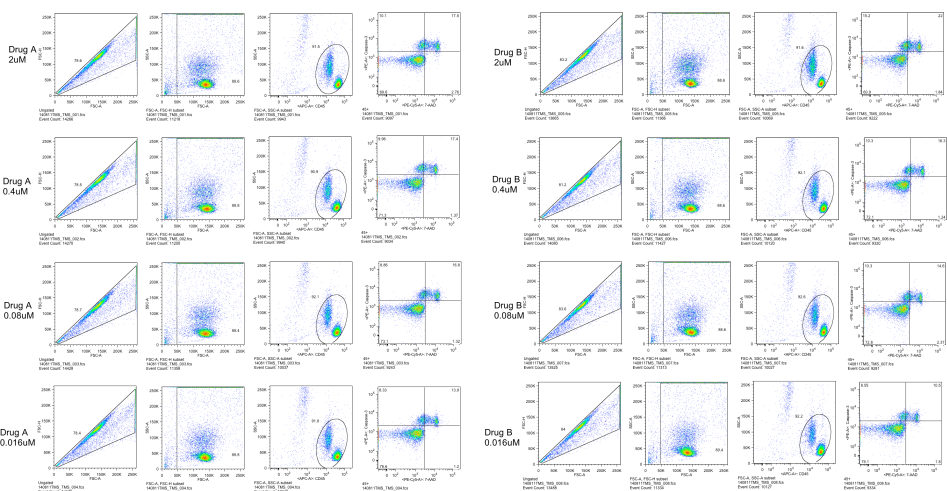
171-017 CLL Only, cont.



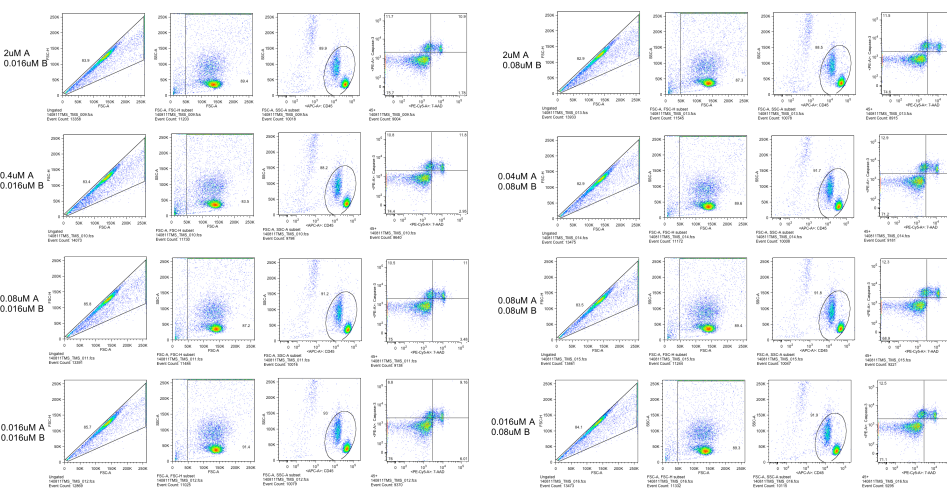
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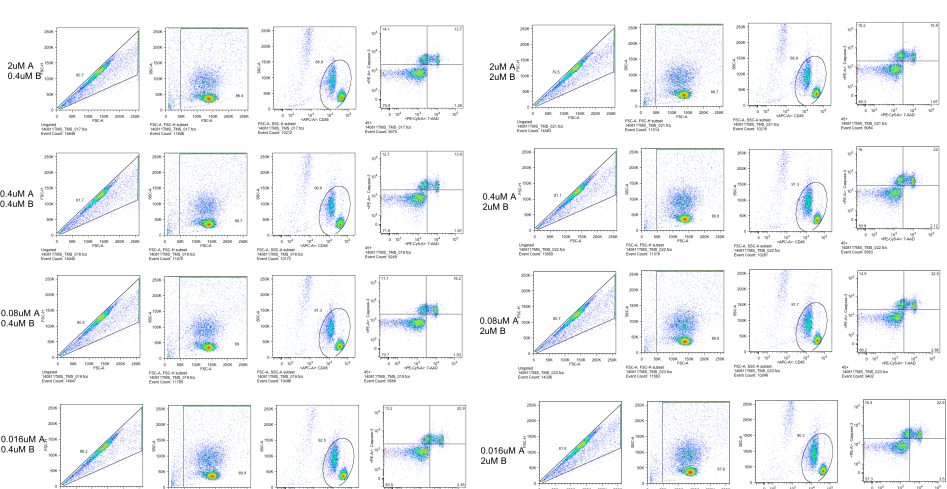
171-017 Stromal Cells



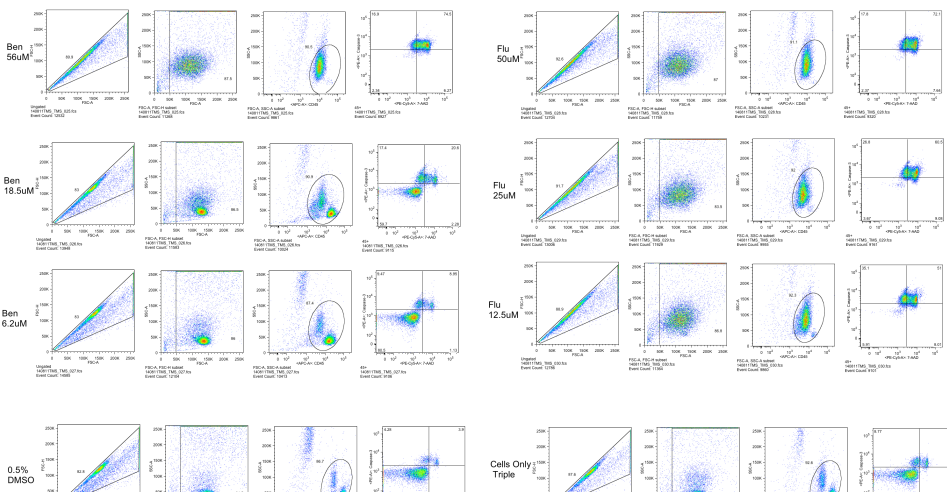
171-017 Stromal Cells, con't.



171-017 Stromal Cells, con't.

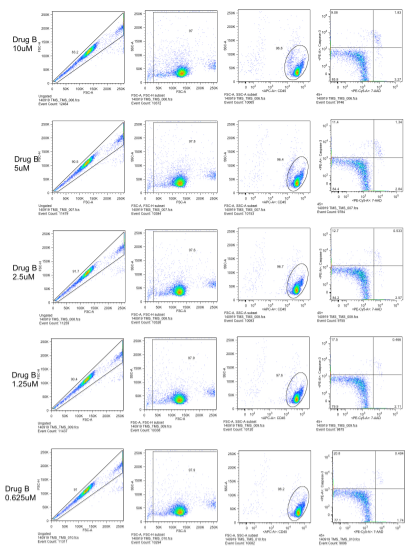
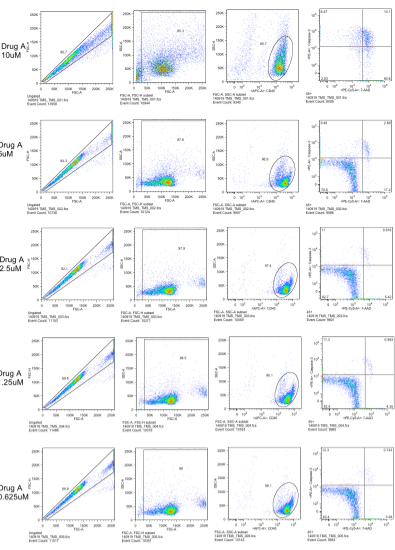


171-017 Stromal Cells, con't.

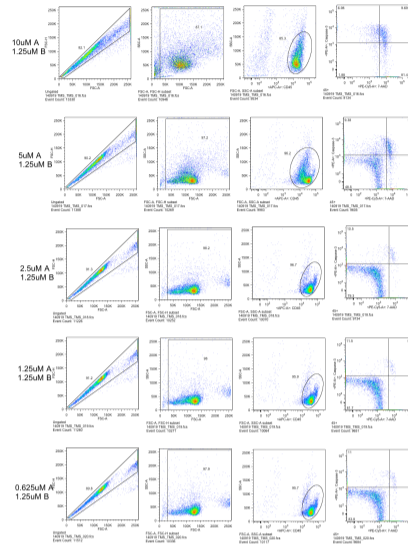
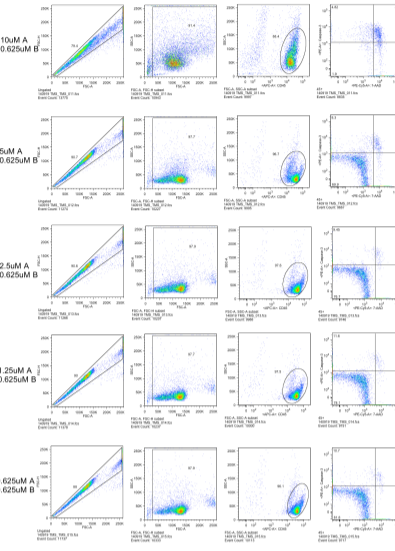


Ibrutinib and trametinib/GSK212 as single agents and in combination treatment of primary CLL cells cultured in media (CLL only) or on stromal cells (Stromal cells) using a flow cytometry assay for drug induced apoptosis and cell death

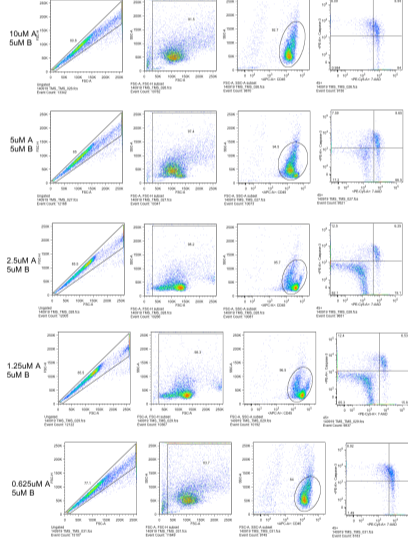
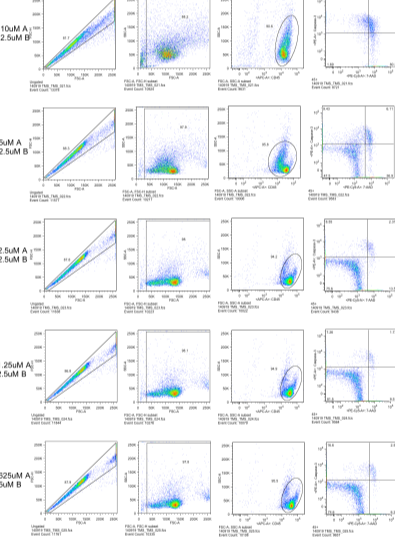
292-013 CLL Cells Only



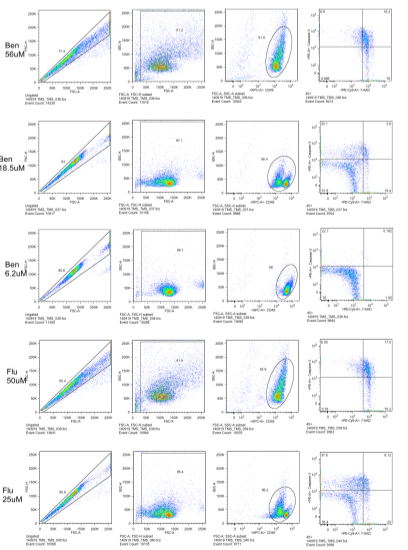
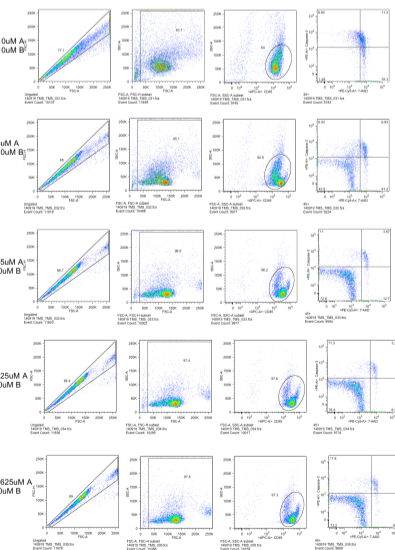
292-013 CLL Cells Only, con't.



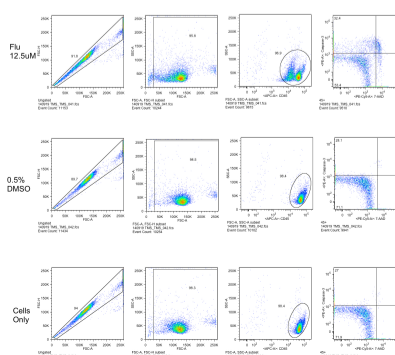
292-013 CLL Cells Only, con't.



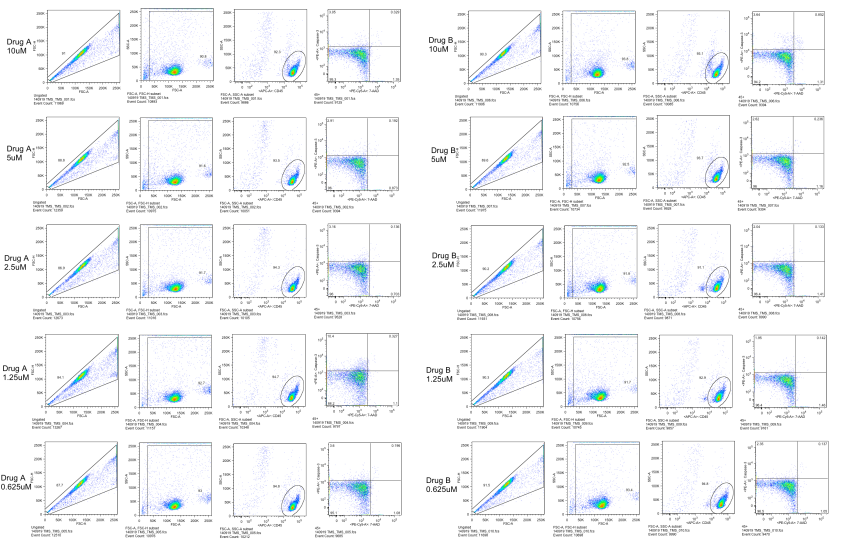
292-013 CLL Cells Only, con't.



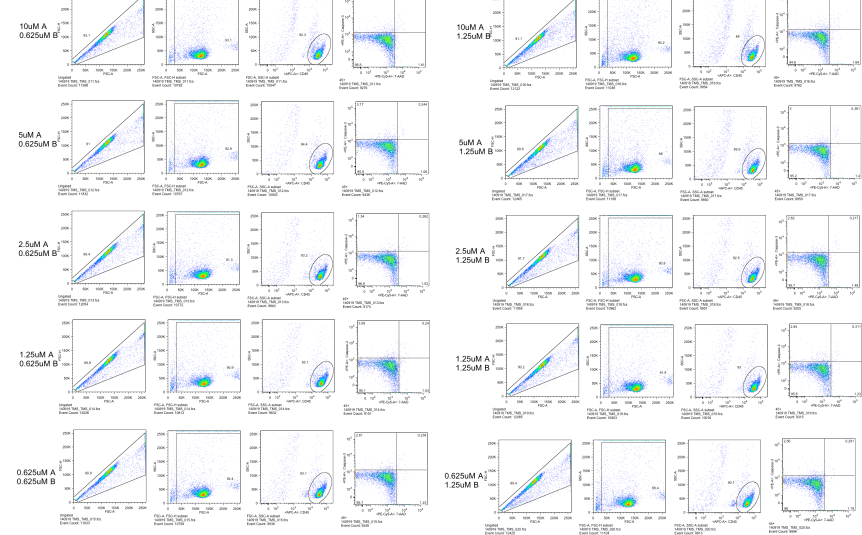
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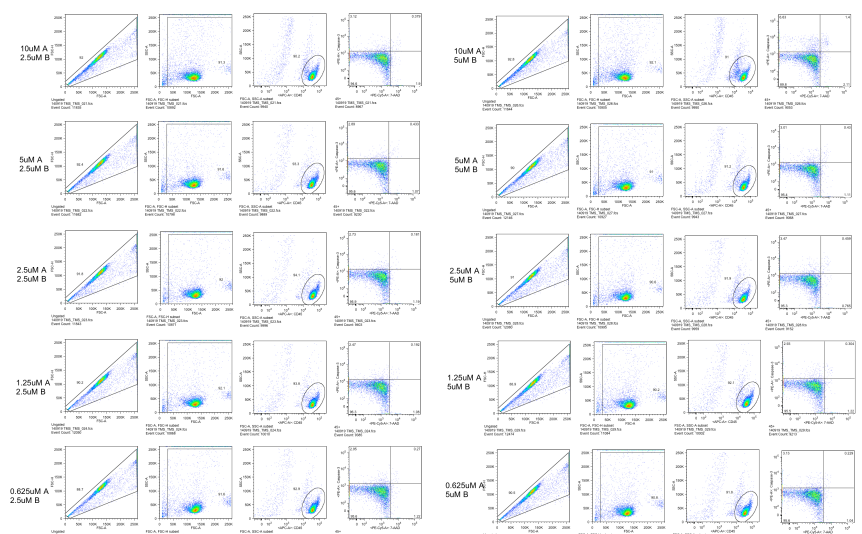
292-013 CLL + Stromal Cells



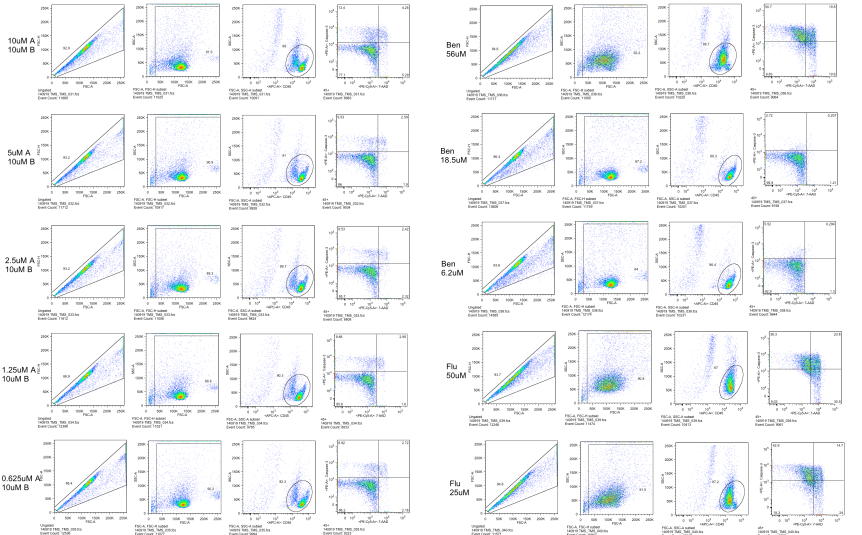
292-013 CLL + Stromal Cells, con't.



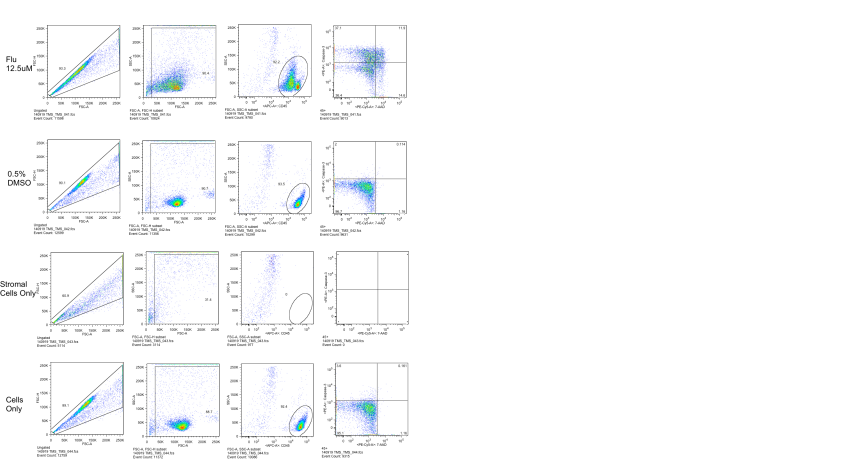
292-013 CLL + Stromal Cells, con't.



292-013 CLL + Stromal Cells, con't.



292-013 CLL + Stromal Cells, con't.



APPENDIX C:

CURRICULUM VITAE Danielle Marie-Underkoffler Brander, MD

Business Address: Duke University Medical Center
2301 Erwin Road
DUMC 3872
Durham, NC 27710
Office Phone: (919) 286 6897
Appointment Phone: (919) 684-8964
Business FAX: (919) 684-5325
E-mail: danielle.brander@dm.duke.edu

Education and Training:

Post-Graduate:

2010-2013	Duke University Medical Center Department of Internal Medicine	Fellow Hematology / Oncology
2007-2010	Duke University Medical Center Department of Internal Medicine Durham, NC	Resident Internal Medicine

Graduate:

2003-2007	School of Medicine Duke University Durham, NC	M.D.
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Undergraduate:

1999-2003	Elizabethtown College Elizabethtown, PA	B.S. in Biology Summa Cum Laude
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Medical Licensure and Certification

2010-Present	American Board of Internal Medicine, Board Certified
2013-	NC State Medical License (unrestricted)
2013-	Board Eligible, Medical Oncology
2013-	Board Eligible, Hematology

Appointments and Positions:

2013-present	Medical Instructor, Division of Hematologic Malignancies & Cellular Therapy, Duke University Medical Center
2010-2013	Fellow, Hematology and Medical Oncology, Duke University Medical Center
2007-2010	Internship and Residency, Internal Medicine, Duke University Medical Center
2005-2006	Howard Hughes Medical Institute (HHMI) Medical Fellow
2001-2003	Research Assistant, Depts. of Biology, Elizabethtown College, Elizabethtown PA & Pomona College, Claremont CA

Membership in Professional and Scientific Societies:

2007-2010	American Medical Association
2010-Present	American Society of Hematology
2014-Present	American Society of Clinical Oncology

Awards and Honors:

1998	HOBYS Youth Leadership Ambassador
1998	Pennsylvania Governor's School for Health Care Scholar
1999	Christian McNaughton PA State Big 33 Dollars for Scholars Scholarship
1999-2003	Elizabethtown College Presidential Scholarship
2000, 2001, 2003	Outstanding Student Award, Elizabethtown College
2001-	Beta Beta Beta & Pi Mu Epsilon Honor Societies, Elizabethtown College
2001	Thomas C Conover Scholarship for Academic Excellence
2001	American Chemical Society Polyed Undergraduate Award in Organic Chemistry
2001-2003	College Scholar, Elizabethtown College
2002	Benjamin G. and Vera B Musser Premedical Scholarship, Elizabethtown College
2005-2006	Howard Hughes Medical Institute (HHMI) Fellowship
2006-2007	Duke University School of Medicine Senior Scholarship Award
2007	Platform presentation AOA Research Day, Duke University School of Medicine
2012-2013	American Society of Hematology Clinical Research Training Institute (ASH CRTI)
2013	Silber Award for Most Outstanding Fellow Research
2013	NCOA/SCOS North Carolina Fellow Recognition for Outstanding Research
2013	Institute for Medical Research Outstanding Poster Presentation Award, 1 st Place

Invited Lectures:

1. From Hydra to Humans: The Evolution of a Research Experience. Young Alumni Guest Lecturer, Elizabethtown College; April 3, 2006.
2. Lenalidomide in Combination with Plerixafor in Patients with Previously Treated Chronic Lymphocytic Leukemia. Young Investigators' Meeting on the occasion of the IXth International Workshop of the German CLL Study Group; September 14th 2012.
3. North Carolina Oncology Association/South Carolina Oncology Society Annual Meeting Invited North Carolina Fellow Presentation for Outstanding Research: Targeting Signaling Pathways in Chronic Lymphocytic Leukemia: Focus on the Lenalidomide and Plerixafor Phase I/Ib Clinical Trial for Patients with Previously Treated CLL. NCOA/SCOS 2013 Meeting, Columbia SC, February 22-23, 2013.
4. The Duke Debates in Hematological Malignancies: Ibrutinib for Frontline CLL Therapy? The Mills House, Charleston, SC, March 14-16th, 2014.

Grand Rounds and Institutional Teaching Presentations:

1. The Bence Jones & the Beans: Pathophysiology and Treatment Considerations in Multiple Myeloma Presenting with Acute Renal Failure. Hematology and Oncology Grand Rounds. October 27th, 2010.
2. Diagnosis and Treatment of Leptomeningeal Metastases in Prostate Cancer and Other Solid Tumor Malignancies. Hematology and Oncology Grand Rounds. March 2, 2011.
3. Prognostic Factors and Risk Stratification in Chronic Lymphocytic Leukemia. Hematology and Oncology Grand Rounds. May 18th, 2011.
4. Malignancy-Associated and Other Thrombotic Microangiopathies (TMAs): Thinking Beyond Thrombotic Thrombocytopenic Purpura (TTP). Hematology and Oncology Grand Rounds. November 9th, 2011.
5. Targeting Signaling Pathways in Chronic Lymphocytic Leukemia. Annual Fellow's Research Retreat. January 27th, 2012.
6. Hairy Cell Leukemia: Case Presentation and Literature Review. Hematology and Oncology Grand Rounds. February 22nd, 2012.
7. Lenalidomide in Combination with Plerixafor in Patients with Previously Treated Chronic Lymphocytic Leukemia: Rationale, Laboratory Correlates, and the CLL Microenvironment. Hematologic Malignancies Grand Rounds. March 9, 2012.
8. T-cell Prolymphocytic Leukemia (T-PLL). Hematology and Oncology Grand Rounds. July 18th, 2012.
9. Research in Progress: Targeting Signaling Pathways in Chronic Lymphocytic Leukemia. Hematology and Oncology Grand Rounds. August 18th, 2012.
10. Tracheal Mass in a Patient with CLL. Hematological Malignancies Grand Rounds. November 30th, 2012.

11. Early Stage Follicular Lymphoma: Can We Really Cure? Hematological Malignancies Grand Rounds. August 2, 2013.
12. Coincidence or Causes: CLL and Pleural Effusions. Hematological Malignancies Grand Rounds. May 16th, 2014.
13. Putting the “I” in Frontline: The future role of ibrutinib and idelalisib as first therapy for patients with CLL with special considerations on toxicities and drug resistance. Hematological Malignancies Grand Rounds. August 22nd, 2014.

Clinical Trials (Principal Investigator)-IRB approved, active or opening:

1. AbbVie: A Phase 1b Study Evaluating The Safety and Tolerability of ABT-199 in Combination with Rituximab in Subjects with Relapsed Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma (M13-365)
2. TG Therapeutics: A Phase I Dose Escalation Study Evaluating the Safety and Efficacy of TGR-1202 in Patients with Relapsed or Refractory Hematologic Malignancies
3. Gilead: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of Idelalisib in Combination with Bendamustine and Rituximab for previously Untreated Chronic Lymphocytic Leukemia

Clinical Trials (Principal Investigator)- non-active:

1. AbbVie: Open-Label Study of the Efficacy of ABT-199 (GDC-0199) in Subjects with Relapsed or Refractory Chronic Lymphocytic Leukemia Harboring the 17p Deletion
2. Gilead: GS-US-339-0103, A Phase 2, Open-Label Study Evaluating the Efficacy, Safety, Tolerability, and Pharmacodynamics of GS 9973 in Combination with Idelalisib in Subjects with Relapsed or Refractory Hematologic Malignancies

Bibliography:

Selected Abstracts, Posters, and Oral Presentations:

1. **Underkoffler DM**, Bean GR, Baker Jr JG, Scott V, Troch MM, Wilke LG, Yee L, Dong M, Marcom PK, Kilmer BF, Fabian CJ, Zalles CM, Broadwater G, and Seewaldt VL: The Frequency of Loss of Heterozygosity of RAR β 2 in Random Periareolar Fine Needle Aspiration Samples of Women at High Risk of Developing Breast Cancer [Abstract]. Duke Comprehensive Cancer Center Annual Meeting; April 10, 2006.
2. **Underkoffler DM** and Seewaldt VL: The Frequency of Loss of Heterozygosity of RAR β 2 in Random Periareolar Fine Needle Aspiration Samples of Women at High Risk of Developing Breast Cancer. [Oral Presentation]. HHMI Meeting of Medical Fellows; May 9-11 2006.
3. **Brander DM** and Seewaldt VL: The Frequency of Loss of Heterozygosity and Microsatellite Instability of RARB and p16INK4A in Atypical Cytology from Women at High-Risk of Developing Breast Cancer [Platform presentation]. Duke University School of Medicine AOA Research Day; September 25, 2006.
4. **Brander DM**, Allgood SD, Matta KM, Weinberg JB, and Lanasa MC: The Role of Surface Immunoglobulin Isotype in CLL Disease Biology and Clinical Outcome [Abstract and Poster Presentation]. American Society of Hematology Annual Meeting; December 9-13th, 2011.
5. **Brander DM**, Allgood SD, Weinberg JB, and Lanasa MC: Lenalidomide in Combination with Plerixafor in Patients with Previously Treated Chronic Lymphocytic Leukemia [Abstract, selected for oral presentation]. Young Investigators' Meeting on the occasion of the IXth International Workshop of the German CLL Study Group; September 14th-16th 2012.
6. **Brander DM**, Beaven AW, Gockerman JP, Diehl LF, Gasparetto C, Shea TC, deCastro C, Moore JO, and Rizzieri DA. Phase II Study of VEGF Inhibitor, PTK787/ZK222584, in Patients with Refractory or Relapsed Diffuse Large B-Cell Lymphoma. [Abstract, Poster Presentation]. American Society of Hematology Annual Meeting; December 8-11th, 2012.
7. Friedman DR, Lanasa MC, **Brander DM**, Allgood SD, Davis ED, Miskin H, Viswanadha S, Vakkalanka S, and Weinberg JB. Comparison of the PI3K- γ Inhibitors TGR-1202 and GS-1101in

- Inducing Cytotoxicity and Inhibiting Phosphorylation of Akt in CLL Cells in vitro. [Abstract]. American Society of Hematology Annual Meeting; December 8-11th, 2012.
8. Lanasa MC, Allgood SD, Goldin L, **Brander DM**, and McMaster ML. Circulating B Cell Clones in Familial Waldenstrom Macroglobulinemia. [Abstract]. American Society of Hematology Annual Meeting; December 8-11th, 2012.
 9. **Brander DM**, Allgood SD, Weinberg JB, and Lanasa MC: The Role of Surface Immunoglobulin Isotype in Chronic Lymphocytic Leukemia Disease Biology and Clinical Outcomes. [Abstract, Poster Presentation, 1st place Award]. 2013 Institute for Medical Research (IMR) Young Investigator Research Symposium; May 15th, 2013.
 10. **Brander DM**, Allgood SD, Simms TM, Weinberg JB, and Lanasa MC: Phase I/Ib Clinical Trial of Lenalidomide and Plerixafor for Patients with Previously Treated Chronic Lymphocytic Leukemia. [Abstract, Poster Presentation]. 1st Annual Duke Cancer Institute Scientific Retreat; May 17th, 2013.
 11. **Brander DM**, Allgood SD, Simms TM, Weinberg JB, and Lanasa MC: Laboratory Correlates and a Phase I Clinical Trial Of Lenalidomide In Combination With Plerixafor In Patients With Previously Treated Chronic Lymphocytic Leukemia. [Abstract]. American Society of Hematology Annual Meeting. December 9-12, 2013.
 12. Burris HA, Patel MR, Lanasa MC, **Brander DM**, O'Connor OA, Deng C, Gutierrez M, Fields Jones S, Kuhn JG, Miskin HP, Sportelli P, Vakkalanka S, Flinn I. TGR-1202, a novel once daily PI3K δ inhibitor, demonstrates clinical activity with a favorable safety profile in patients with relapsed or refractory hematologic malignancies. [Abstract 2513, Poster]. American Society of Clinical Oncology Annual Meeting. May 30-June 3, 2014.
 13. Ma S, **Brander DM**, Kipps TJ, Barrientos JC, Davids MS, Mason-Bright T, Rudersdorf N, Yang J, Munasinghe W, Zhu M, Cerri E, Enschede SH, Humerickhouse RA, Roberts AW. ABT-199 (GDC-0199) Combined with Rituximab (R) in Patients (pts) With Relapsed / Refractory (R/R) Chronic Lymphocytic Leukemia (CLL): Interim Results of a Phase 1b Study. [Abstract 7013, Poster]. American Society of Clinical Oncology Annual Meeting. May 30-June 3, 2014.
 14. Roberts AW, Ma S, **Brander DM**, Kipps TJ, Barrientos JC, Davids MS, Mason-Bright T, Rudersdorf N, Yang J, Munasinghe W, Zhu M, Cerri E, Enschede SH, Humerickhouse RA, Seymour JF. ABT-199 (GDC-0199) Combined with Rituximab (R) in Patients (pts) With Relapsed / Refractory (R/R) Chronic Lymphocytic Leukemia (CLL): Interim Results of a Phase 1b Study. [Abstract S703, Oral Presentation]. 19th Congress of the European Hematology Association. June 14, 2014.
 15. Burris HA, Patel MR, Lanasa MC, **Brander DM**, O'Connor OA, Deng C, Gutierrez M, Fields Jones S, Kuhn JG, Miskin HP, Sportelli P, Vakkalanka S, Flinn I. TGR-1202, a novel once daily PI3K δ inhibitor, demonstrates clinical activity with a favorable safety profile in patients with relapsed or refractory hematologic malignancies. [Abstract P250, Poster]. 19th Congress of the European Hematology Association. June 13, 2014.
 - 16.

Publications:

1. Bean GR, Bryson AD, Pillie PG, Goldenberg V, Baker JC, Ibarra C, **Brander DM**, Paisie C, Case NR, Gauthier M, Reynolds PA, Dietze E, Ostrander J, Scott V, Wilke LG, Yee L, Zalles CM, Broadwater G, Tistry TD, Seewaldt VL. Morphologically Normal-Appearing Mammary Epithelial Cells Obtained from High-Risk Women Exhibit Methylation Silencing of INK4a/ARF. Clin Cancer Res. 2007; 13:6834-6841. [PMID: 18006786]
2. Bridge DM, Ha CT, Nemir A, Renden A, Rorick MM, Shaffer AL, **Underkoffler DM**, Willis AE, Martinez DE. (2004) Variations on a theme? Polyp and medusa development in Podocoryna carnea. Hydrobiologia. 530: 299-307.
3. **Brander DM** and Beaven AW. Update on the Treatment of Follicular Non-Hodgkin Lymphoma-Focus on the Potential of Bortezomib. Patient Prefer Adherence. 2012;6:239-51. [PMID: 22536060]
4. **Brander DM**, Rizzieri D, Gockerman J, Diehl L, Shea TC, Decastro C, Joseph O, Moore JO, and Beaven AW. Phase II Open Label Study of the oral VEGF-Receptor inhibitor PTK787/ZK222584 (Vatalanib) in Adult Patients with Refractory or Relapsed Diffuse Large B Cell Lymphoma. Leukemia & Lymphoma. Published online on March 14, 2013. [PMID: 23488610]
5. Friedman DR, Lanasa MC, Davis PH, Allgood SD, Matta KM, **Brander DM**, Davis ED, Volkheimer AD, Moore JO, Gockerman JP, Sportelli P, Weinberg JB. Perifosine Treatment in Chronic Lymphocytic

Leukemia: Results of a Phase II Clinical Trial and In Vitro Studies. Leuk Lymphoma. Published Online July 17, 2013. [PMID:23863122]

Other Support [Funded]:

“Understanding Drug Resistance to Targeted Therapeutics in Malignant B-Cell Lymphoproliferative Disorders (B-LPDs)” [Awarded]

PI: Danielle M. Brander

9/15/2013-9/15/2016

Agency: Department of Defense

NIH Loan Repayment Program Clinical Research Scholar. “Understanding the Tumor Microenvironment as a Mechanism of Drug Resistance and Potential Therapeutic Target for Malignant B-cell Lymphoproliferative Disorders (B-LPDs)”

PI: Danielle M. Brander

7/1/2013-6/30/2015

Agency: NIH

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Friedman, Daphne Ruth	POSITION TITLE Assistant Professor, Medical Oncology
eRA COMMONS USER NAME (credential, e.g., agency login): DAPHNE.FRIEDMAN	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Brown University, Providence RI	Sc.B.	05/98	Biology
University of Maryland, Baltimore MD	M.D.	05/02	Medicine
University of Maryland, Baltimore MD	Residency	06/05	Internal Medicine
Duke University, Durham NC	Fellowship	06/08	Hematology/Oncology

A. Personal Statement

My research goals involve improving clinical care and outcomes of patients with chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphomas (NHL). This includes work to develop and evaluate prognostic and predictive biomarkers in CLL and NHL, such as multivariate models of clinically utilized prognostic markers, gene expression profiling signatures, and expression of endogenous inhibitors of protein phosphatase 2A (PP2A). Other work has involved preclinical and clinical evaluation of novel targeted therapeutics in these malignancies (e.g. LMP-420, perifosine, TG-1202). Together with my clinical interest in providing excellent patient care, these research projects aim to positively impact the clinical management of patients with lymphoid malignancies.

B. Positions and Honors

Position and Employment

2005-2008 Fellow, Hematology/Oncology, Duke University Medical Center, Durham, NC
2008-2013 Instructor, Medical Oncology, Duke University Medical Center, Durham, NC
2013- Assistant Professor, Medical Oncology, Duke University Medical Center, Durham, NC
2013- Physician, Durham VA Medical Center, Durham, NC

Other Experience and Professional Memberships

2003- Member, American College of Physicians
2005- Member, American Society of Hematology
2006- Member, American Society of Clinical Oncology
2006 Fellow at the Molecular Biology in Clinical Oncology AACR Workshop
2008- Associate Member, Duke Cancer Institute

Honors

2002 American Medical Women's Association Janet M. Glasgow Memorial Achievement Citation
2002 Alpha Omega Alpha, University of Maryland
2007 American Society of Clinical Oncology Young Investigator Award
2008 Barton F. Haynes Research Award, Duke University
2008 Young Investigator Award, Duke Comprehensive Cancer Center Annual Meeting

C. Peer-reviewed Publications

Most relevant (in chronological order)

1. Weinberg JB, Volkheimer AD, Chen Y, Beasley BE, Jiang N, Lanasa MC, Friedman D, Vaccaro G, Rehder C, DeCastro CM, Rizzieri DA, Diehl LF, Gockerman JP, Moore JO, Goodman BK, Levesque MC. "Clinical and molecular predictors of disease severity and survival in chronic lymphocytic leukemia." *American Journal of Hematology*. 2007; 82(12): 1063-70. PMC2783430.
2. Zhang J, Jima DD, Jacobs C, Fischer R, Gottwein E, Huang G, Lugar PL, Lagoo AS, Rizzieri DA, Friedman DR, Weinberg JB, Lipsky PE, Dave SS. "Patterns of microRNA expression characterize stages of human B-cell differentiation." *Blood*. 2009; 113(19): 4586-94. PMC2680365.
3. Friedman DR, Weinberg JB, Barry WT, Goodman BK, Volkheimer AD, Bond KM, Chen Y, Jiang N, Moore JO, Gockerman JP, Diehl LF, Decastro CM, Potti A, Nevins JR. "A Genomic Approach to Improve Prognosis and Predict Therapeutic Response in Chronic Lymphocytic Leukemia." *Clinical Cancer Research*. 2009; 15(22): 6947-55. PMC2783430.
4. Mowery YM, Weinberg JB, Kennedy MN, Bond KM, Moore JO, Lanasa MC, Gockerman JP, Diehl LF, Pizzo SV, Cianciolo GJ, Friedman DR. "LMP-420: a novel purine nucleoside analogue with potent cytotoxic effects for CLL cells and minimal toxicity for normal hematopoietic cells." *Leukemia*, 2010; 24(9): 1580-7. PMID: 20613784.
5. Jima DD, Zhang J, Jacobs C, Richards KL, Dunphy CH, Choi WW, Yan Au W, Srivastava G, Czader MB, Rizzieri DA, Lagoo AS, Lugar PL, Mann KP, Flowers CR, Bernal-Mizrachi L, Naresh KN, Evens AM, Gordon LI, Luftig M, Friedman DR, Weinberg JB, Thompson MA, Gill JI, Liu Q, How T, Grubor V, Gao Y, Patel A, Wu H, Zhu J, Blobe GC, Lipsky PE, Chadburn A, Dave SS. "Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs." *Blood*, 2010; 116(23): e118-e127. PMC3012600.
6. Friedman DR, Magura LA, Warren HA, Harrison JD, Diehl LF, Weinberg JB. "Statin use and the need for therapy in chronic lymphocytic leukemia." *Leuk Lymphoma*. 2010; 51(12): 2295-8. PMID: 20929315.
7. Christensen DJ, Chen Y, Oddo J, Matta KM, Neil J, Davis ED, Volkheimer AD, Lanasa MC, Friedman DR, Goodman BK, Gockerman JP, Diehl LF, de Castro CM, Moore JO, Vitek MP, Weinberg JB. "SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin's lymphoma: a predictor of aggressive disease and new treatment target." *Blood*. 2011;118(15): 4150-8. PMC3204732.
8. Lanasa MC, Davis PH, Datto M, Li Z, Gockerman JP, Moore JO, Decastro CM, Friedman DR, Diehl LF, Rehder C, Cook H, Daugherty FJ, Matta KM, Weinberg JB, Rizzieri D. "Phase II Study of Cenersen, an Antisense Inhibitor of p53, in Combination with Fludarabine, Cyclophosphamide, and Rituximab for High Risk CLL." *Leuk Lymphoma*. 2012; 53(2): 218-24. PMID: 21827374.
9. Dilillo DJ, Weinberg JB, Yoshizaki A, Horikawa M, Bryant JM, Iwata Y, Matsushita T, Matta KM, Chen Y, Venturi GM, Russo G, Gockerman JP, Moore JO, Diehl LF, Volkheimer AD, Friedman DR, Lanasa MC, Hall RP, Tedder TF. "Chronic lymphocytic leukemia and regulatory B cells share IL-10-competence and immunosuppressive function." *Leukemia*. 2013. 27:170-82. PMID: 22713648.
10. Friedman DR, Lucas JE, Weinberg JB. "Clinical and Biological Relevance of Genomic Heterogeneity in Chronic Lymphocytic Leukemia." *PLOS One*. 2013. 8(2): e57356. PMID: 23468975. PMC3585365.
11. Nipp RD, Volkheimer AD, Davis ED, Chen Y, Weinberg JB, Friedman DR. "CD38 variation as a prognostic factor in chronic lymphocytic leukemia." *Leuk Lymphoma*. 2013. Epub ahead of print. PMID: 23510236.
12. Friedman DR, Lanasa MC, Davis PH, Allgood SD, Matta KM, Brander DM, Chen Y, Davis ED, Volkheimer AD, Moore JO, Gockerman JP, Sportelli P, Weinberg JB. "Perifosine treatment in chronic lymphocytic leukemia: Results of a phase II clinical trial and *in vitro* studies." *Leuk Lymphoma*. 2013. Epub ahead of print. PMID: 23863122.

Additional peer-reviewed publications (in chronological order)

1. Vazquez N, Walsh TJ, Friedman D, Chanock SJ, Lyman CA. "Interleukin-15 augments superoxide production and microbicidal activity of human monocytes against Candida albicans." Infect Immun. 1998; 66(1): 145-150. PMC107870.
2. Friedman DR, Wolfsthal SM. "Cocaine-Induced Pseudovasculitis." Mayo Clin Proc. 2005; 80(5): 671-673. PMID: 15887436.
3. Friedman DR, Coan A, Smith SK, Herndon JE II, Abernethy AP. "Informational needs assessment of non-Hodgkin lymphoma survivors and their physicians." American Journal of Hematology. 2010; 85(7): 528-532. PMC3160829.

Relevant non-peer reviewed publications (in chronological order)

1. Friedman DR, "'Primum non nocere": the addition of granulocyte-macrophage colony stimulating factor to alemtuzumab in chronic lymphocytic leukemia." Leuk Lymphoma. 2013. 54:441-442. PMID: 22950412.
2. Friedman DR, Weinberg JB. "Inhibition of B cell receptor signaling as a therapeutic strategy for treatment of chronic lymphocytic leukemia." The Hematologist. 2013. 10:5-7.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME J. Brice Weinberg	POSITION TITLE Professor of Medicine and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login) weinb001	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Univ Arkansas, Fayetteville, AR	BS	06/66	Science
Univ Arkansas Sch of Medicine, Little Rock, AR	MD	06/69	Medicine
Univ Hospital, Univ Arkansas, Little Rock, AR	Resident	06/69-09/71	Medicine
Univ of Utah Medical School, Salt Lake City, UT	Fellow	09/73-06/76	Hematology-Oncology

A. Personal statement.

I am a research scientist and physician (internal medicine and hematology-oncology) who has worked since 1969 in clinical medicine and since 1974 in medical research with the intent of translating laboratory research findings to use in clinical medicine. Over the last 12 years, I have placed special emphasis on studies of chronic lymphocyte leukemia (CLL). We longitudinally study approximately 615 CLL patients relative to CLL pathogenesis, genetics, and epidemiology. Our work has identified novel targets for therapy in CLL, and we have translated our laboratory studies into ongoing clinical studies related to biomarkers & prognostication, and trials of new treatments for CLL.

B. Recent positions and honors

- Associate Chief of Staff for Education, VA Medical Center, Durham, NC 1997-1999.
- Associate Prof. of Obstetrics and Gynecology, Duke Univ School of Medicine, Durham, NC 1995-present.
- Prof. of Medicine, Duke Univ School of Medicine, Durham, NC 1990-present.
- Prof. of Immunology, Duke Univ School of Medicine, Durham, NC, 1997-present.
- Associate Chief of Staff for Research and Development, VA Medical Center, Durham, NC 2000-2006
- Associate Investigator, Salt Lake City VA Hospital, Salt Lake City, Utah, 1976-1978
- Staff Physician, VA Medical Center, Durham, NC, 1978-present
- Member, Southeast Cancer Chemotherapy Study Group, 1978-986
- Director, Durham County Hospice, Durham, NC, July 1984-July 1986, Member
- Cancer and Leukemia Group B, 1986 to present, Director, VA District 8 Cancer Network, 1987-1990
- Medical Investigator, Durham VA Medical Center, Durham, NC, 1991-1997
- Associate Chief of Staff for Education, VA Medical Center, Durham, NC 1996-1999
- Chief of Hematology-Oncology, VA Medical Center, Durham, NC, 1982-1990, 1996-1997, and 1999-2000
- Chairman, VA VISN 6 Research Subcouncil, Durham, NC, 2000-2006
- President, Institute of Medical Research, Durham, NC 2000-2006
- Associate Chief of Staff for Research, VA Medical Center, Durham, NC 2000-2006

Honors & other special scientific recognition (selected):

Phi Beta Kappa	Outstanding Med Resident	Amer Soc of Hematology
Amer Soc for Clin Invest	Assoc Amer Physicians	Duke Univ Wendell Rosse Teaching Award
Duke Univ Davison Soc Excellence in Teaching Award	NIH Lifetime Achievement Award for Nitric Oxide Research ('08)	VA Middleton Award: Highest Achievement in Research ('11)

C. Selected peer-reviewed publications (leukemia-related manuscripts from more than 260 publications):

1. Friedman DR, Weinberg JB, Barry WT, Goodman BK, Volkheimer AD, Bond KM, Chen Y, Jiang N, Moore JO, Gockerman JP, Diehl LF, Decastro CM, Potti A, Nevins JR. A genomic approach to improve prognosis and predict therapeutic response in chronic lymphocytic leukemia. *Clin Cancer Res* 15: 6947-6955, 2009. PMC2783430.
2. Conde L, Halperin E, Akers NK, Brown KM, Smedby KE, Rothman N, Nieters A, Slager SL, Brooks-Wilson A, Agana L, Riby J, Liu J, Adami HO, Darabi H, Hjalgrim H, Low HQ, Humphreys K, Melbye M, Chang ET, Glimelius B, Cozen W, Davis S, Hartge P, Morton LM, Schenk M, Wang SS, Armstrong B, Krickler A, Milliken S, Purdue MP, Vajdic CM, Boyle P, Lan Q, Zahm SH, Zhang Y, Zheng T, Becker N, Benavente Y, Boffetta P, Brennan P, Butterbach K, Cocco P, Foretova L, Maynadie M, de Sanjose S, Staines A, Spinelli JJ, Achenbach SJ, Call TG, Camp NJ, Glenn M, Caporaso NE, Cerhan JR, Cunningham JM, Goldin LR, Hanson CA, Kay NE, Lanasa MC, Leis JF, Marti GE, Rabe KG, Rassenti LZ, Spector LG, Strom SS, Vachon CM, Weinberg JB, Holly EA, Chanock S, Smith MT, Bracci PM, Skibola CF. Genome-wide association study of follicular lymphoma identifies a risk locus at 6p21.32. *Nat Genet* 42: 661-664, 2010. PMC2913472.
3. Lanasa MC, Allgood SD, Volkheimer AD, Gockerman JP, Whitesides JF, Goodman BK, Moore JO, Weinberg JB, Levesque MC. Single-cell analysis reveals oligoclonality among 'low-count' monoclonal B-cell lymphocytosis. *Leukemia* 24: 133-140, 2010. PMC2806490.
4. Mowery YM, Weinberg JB, Kennedy MN, Bond KM, Moore JO, Lanasa MC, Gockerman JP, Diehl LF, Pizzo SV, Cianciolo GJ, Friedman DR. LMP-420: a novel purine nucleoside analog with potent cytotoxic effects for CLL cells and minimal toxicity for normal hematopoietic cells. *Leukemia* 24: 1580-1587, 2010. PMID: 20613784.
5. Lanasa MC, Allgood SD, Slager SL, Dave SS, Love C, Marti GE, Kay NE, Hanson CA, Rabe KG, Achenbach SJ, Goldin LR, Camp NJ, Goodman BK, Vachon CM, Spector LG, Rassenti LZ, Leis JF, Gockerman JP, Strom SS, Call TG, Glenn M, Cerhan JR, Levesque MC, Weinberg JB, Caporaso NE. Immunophenotypic and gene expression analysis of monoclonal B-cell lymphocytosis shows biologic characteristics associated with good prognosis CLL. *Leukemia*, 25:1459-1466, 2011. PMC3164475.
6. Christensen DJ, Chen Y, Oddo J, Matta KM, Neil J, Davis ED, Volkheimer AD, Lanasa MC, Friedman DR, Goodman BK, Gockerman JP, Diehl LF, de Castro CM, Moore JO, Vitek MP, Weinberg JB. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin's lymphoma: a predictor of aggressive disease and new treatment target. *Blood* 118:4150-4158, 2011. PMC3204732.
7. Coombs CC, Rassenti L, Falchi L, Slager SL, Strom S, Ferrajoli A, Weinberg JB, Kipps TJ, Lanasa MC. Differential genetic risk among African Americans for common SNPs conferring risk of chronic lymphocytic leukemia. *Blood*, 120:1687-1690, 2012. PMC3429309.
8. Slager SL, Skibola CF, Di Bernardo MC, Conde L, Broderick P, McDonnell SK, Goldin LR, Croft N, Holroyd A, Harris S, Riby J, Serie DJ, Kay NE, Call TG, Bracci PM, Halperin E, Lanasa MC, Cunningham JM, Leis JF, Morrison VA, Spector LG, Vachon CM, Shanafelt TD, Strom SS, Camp NJ, Weinberg JB, Matutes E, Caporaso NE, Wade R, Dyer MJ, Dearden C, Cerhan JR, Catovsky D, Houlston RS. Common variation at 6p21.31 (BAK1) influences the risk of chronic lymphocytic leukemia. *Blood*, 120:843-846, 2012. PMC3412347.
9. DiLillo DJ, Weinberg JB, Iwata Y, Matsushita T, Matta KM, Venturi GM, Russo G, Gockerman JP, Moore JO, Diehl LF, Volkheimer AD, Friedman DR, Lanasa MC, Hall RP, Tedder TF. Chronic lymphocytic leukemia and regulatory B10 cells share a common cellular origin in mice and humans. *Leukemia* 27:10-182, 2013. PMID: 22713648.
10. Coombs CC, Rassenti L, Falchi L, Slager SL, Strom S, Ferrajoli A, Weinberg JB, Kipps TJ, Lanasa MC. Differential genetic risk among African Americans for common SNPs conferring risk of chronic lymphocytic leukemia. *Blood* 120:1687-90, 2012. PMID:22745306.
11. Friedman DR, Lucas JE, Weinberg JB. Clinical and biological relevance of genomic heterogeneity in chronic lymphocytic leukemia. *PLoS One* 8:e57356, 2013. PMID 23468975.
12. Nipp RD, Volkheimer AD, Davis ED, Chen Y, Weinberg JB, Friedman DR. CD38 variation as a prognostic factor in chronic lymphocytic leukemia. *Leuk Lymph*, In press, 2013. PMID 23510236.

13. Berndt SI, CF Skibola Vijai J, Camp NJ, Weinberg JB, (*several other authors...*), Slager S. Genome-wide Association Study Identifies Multiple Loci Associated with Chronic Lymphocytic Leukemia. *Nature Genetics*, In press, 2013. PMID 23770605.
14. Friedman DR, Lanasa MC, Davis PH, Allgood SD, Matta KM, Brander DM, Chen Y, Davis ED, Volkheimer AD, Moore JO, Gockerman JP, Sportelli P, Weinberg JB. Pre-clinical and phase II clinical trial results of perfosine treatment in chronic lymphocytic leukemia. *Leuk Lymph*, In press, 2014
15. Slager SL, Lanasa MC, Kay NE, Goldin LR, Achenbach SJ, Allgood S, Norman A, Vachon CM, Leis JF, Weinberg JB, Rabe KM, Call TG, Cerhan JR, Shanafelt TD, Caporaso NE, Hanson CA. Longitudinal evaluation of monoclonal B-cell lymphocytosis (MBL) among high-risk CLL family members. *Leukemia*, In press, 2014

D. Relevant ongoing research support

1. NIH R01-AI041764: Arginine, Nitric Oxide and Severe Malaria. J.B. Weinberg (PI); 08/01/2008-07/31/2014. *Evaluation of the roles of arginine, nitric oxide, and endothelial function in severe malaria.*
2. Lymphoma Research Foundation of America. T. Tedder (PI); 09/30/2011–09/29/2014. *Molecular Insights into MCL Immunotherapy.*
3. VA Merit Review: Biologic Scaffold Prostheses to Enhance Meniscus Repair. J.B. Weinberg (PI); 10/01/2010-09/30/2014. *Biomechanical and biological evaluations of novel meniscus prostheses.*
4. VA Middleton Award Grant. Funds accompanying VA Middleton Award for Highest Achievement in VA Biomedical Research. J.B. Weinberg (PI); 7/1/2011-06/30/2014. *These are unrestricted funds for biomedical research.*

Completed research support (last 3 years)

1. The Biomarker Factory, LLC. Novel and useful biomarkers in CLL and MBL. 05/01/2011-04/30/2013. J.B. Weinberg (PI). *Studies of biomarkers to predict CLL disease progression and to predict specific drug sensitivity*
2. Leukemia & Lymphoma Society Translational Research Grant: CLL and nitric oxide; J.B. Weinberg (PI); 10/1/05-9/30/10. *Studies of nitric oxide and nitric oxide synthases in the pathogenesis of chronic lymphocytic leukemia.*
3. NIH 1R43-CA137941: Novel targeted therapy in CLL. D.L. Christensen (PI); 7/1/08-12/31/10. *Studies of the role of SET in CLL.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME David A. Rizzieri	POSITION TITLE Professor of Medicine		
eRA COMMONS USER NAME (credential, e.g., agency login) Rizzi003			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Hobart College, Geneva NY	BS	1983 - 1987	General Biology
University of Rochester School of Medicine and Dentistry, Rochester NY	MD	1987 - 1991	M.D.

A. Personal Statement:

As a translational researcher, I have focused my efforts on developing new therapies for patients with leukemia or lymphoma. I am a member of the Combined International Bone Marrow Transplant Registry Lymphoma Writing Committee and am a member of the ALLIANCE (formerly CALGB) Leukemia Core Committee and NCI Leukemia Working Groups. I serve as chair or co-chair of multiple ALLIANCE lymphoma/ leukemia trials, the most recent being the last 2 national studies for Burkitt's lymphoma, as well as the current leukemia in those under 60 years of age intergroup study. I have led our team's novel approaches for the care of patients using monoclonal antibodies with non-myeloablative allogeneic therapy to optimize a less toxic regimen for transplant of patients with hematologic malignancies using haplo-identical, as well as matched donors. This work is currently being extended with post-transplant graft manipulation studies focused on manipulating Natural Killer cell activity. I have led the development of anti-stromal therapy in lymphoma using new antibodies targeting the stromal protein, tenascin, for which a patent is pending, and first in human studies targeting acute myelogenous leukemia by linking diphtheria to an antibody to the IL-3 receptor (now in phase 3). Further, I serve on various NIH/NHLBI grant review committees and am a member of ASH, ASCO, AACR, ASBMT, and ASCI. I oversee the development and conduct of all clinical trials in adults with hematologic malignancies conducted in the Duke Cancer Institute. In recognition of this leadership and success in our clinical research efforts, I was fortunate to receive the NIH 'Clinical Investigator Team Leadership Award' in 2010 and was awarded by the fellows the 'Wendell Rosse Teaching Award' in 2009.

B. Positions and Honors:

1991-1994 Intern and Resident: Strong Memorial Hospital, Univ of Rochester, Rochester, NY
1994-1997 Fellowship: Duke University Medical Center (DUMC), Duke University, Durham, NC
7/97-7/98 Associate in Med., Div of Oncology, DUMC
7/98-7/03 Assistant Professor in Medicine, Div of Oncology and Transplantation, DUMC
7/03-4/13 Associate Professor in Medicine, Div of Cellular Therapy, DUMC (tenure granted '07)
5/13-present Professor of Medicine, Div of Hematologic Malignancies and Cellular Therapy, DUMC
7/97-6/08 Medical Director of Duke Bone Marrow Transplantation Adult Inpatient Unit, DUMC
12/01-12/11 Director of Hematologic Malignancy Program and Cellular Therapy Clinical Research
12/11-present Associate Director for Clinical Research, Div of Hematologic Malignancies and Cellular Therapy
01/13-present Chief, Section of Hematologic Malignancies

Honors / Committees

1987 Phi Beta Kappa, Summa Cum Laude graduate with highest honors, Hobart College
1994 Lawrence E. Young Medical Resident Award- University of Rochester
7/97 – present Cancer Center Protocol Review Committee
7/97 - present Producer/Director of CME cat 1 Hematologic Malignancy Tumor Board, BMT conference and annual Heme Malignancy conference- 'Duke Debates'
Scientific Co-Chair CALGB 50002- Thalidomide for low grade lymphoma
Scientific Co-Chair CALGB 10105- Phase II trial of PTK 787 for MDS
Chair CALGB 10002 - Intense chemotherapy and Rituximab for Burkitt's
Chair- CALGB 9251- Intense therapy for SNC NHL/L3 ALL

1999 Lisa Stafford Young Investigator's Award- Duke University
 2005 Producer: <http://hememalignancy.mc.duke.edu>
 1/05 - present Duke Medical Oncology Clinical Research Leadership Group
 4/05 - present Duke University Medical Center IRB
 4/09 American Society of Clinical Investigation- elected 4/09
 2009 The Wendell Rosse Teaching Award
 2010 – present CALGB (now ALLIANCE) Leukemia Core Committee
 2010 – present NCI Leukemia Working Group
 2011 Division of Cellular Therapy First Annual Teaching Award

C. Selected Peer-reviewed Publications from over 125

1. **Rizzieri DA**, Koh LP, Long GD, Gasparetto C, Sullivan KM, Horwitz M, Chute J, Smith C, Gong JZ, Lagoo A, Niedzwiecki D, Dowell JM, Waters-Pick B, Liu C, Marshall D, Vredenburgh JJ, Gockerman J, Decastro C, Moore J, Chao NJ. Partially matched, nonmyeloablative allogeneic transplantation: clinical outcomes and immune reconstitution. *J Clin Oncol*. 2007 Feb 20;25(6):690-7. Epub 2007 Jan 16. PMID:17228020
2. Adams DJ, Sandvold ML, Myhren F, Jacobsen TF, Giles F, **Rizzieri DA**. Anti proliferative activity of ELACY (CP-4055) in combination with cloretazine (VNP40101M), idarubicin, gemcitabine, irinotecan and topotecan in human leukemia and lymphoma cells. *Leuk Lymphoma*. 49:786-97. 2008. PMID: PMC2562023
3. Smith SM, Grinblatt D, Johnson JL, Niedzwiecki D, **Rizzieri D**, Bartlett NL, Cheson BD; Cancer and Leukemia Group B. Thalidomide has limited single-agent activity in relapsed or refractory indolent non-Hodgkin lymphomas: a phase II trial of the Cancer and Leukemia Group B. *Br J Haematol*. 140:313-9. 2008. PMID: 18217897
4. Lee SJ, Kukreja M, Wang T, Giralt SA, Szer J, Arora M, Woolfrey AE, Cervantes F, Champlin RE, Gale RP, Halter J, Keating A, Marks DI, McCarthy PL, Olavarria E, Stadtmauer EA, Abecasis M, Gupta V, Khoury HJ, George B, Hale GA, Liesveld JL, **Rizzieri DA**, Antin JH, Bolwell BJ, Carabasi MH, Copelan E, Ilhan O, Litzow MR, Schouten HC, Zander AR, Horowitz MM, Maziarz RT. Impact of prior imatinib mesylate on the outcome of hematopoietic cell transplantation for chronic myeloid leukemia. *Blood*. 2008. 112:3500-7. PMID: PMC2954751
5. Frankel A, Liu JS, **Rizzieri D**, Hogge D. Phase I clinical study of diphtheria toxin-interleukin 3 fusion protein in patients with acute myeloid leukemia and myelodysplasia. *Leuk Lymphoma*. 49:543-53. 2008. PMID: 18297533
6. **Rizzieri DA**, Dev P, Long GD, Gasparetto C, Sullivan KM, Horwitz M, Chute J, Chao NJ. Response and toxicity of donor lymphocyte infusions following T-cell depleted non-myeloablative allogeneic hematopoietic SCT from 3-6/6 HLA matched donors. *Bone Marrow Transplant*. 2009 43:327-33. PMID: 18850014
7. **Rizzieri DA**, Feldman E, Dipersio JF, Gabrail N, Stock W, Strair R, Rivera VM, Albitar M, Bedrosian CL, Giles FJ. A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res*. 2008. 14:2756-62. PMID: 18451242
8. **David A. Rizzieri**, Robert Storms, Dong-Feng Chen, Gwynn Long, Daniel A. Nikcevich, Cristina Gasparetto, Mitchell Horwitz, John Chute, Keith Sullivan, Therese Hennig, Debashish Misra, Christine Apple, Megan Baker, Ashley Morris, Patrick G. Green, Vic Hasselblad, Nelson J. Chao. Natural Killer Cell Enriched Donor Lymphocyte Infusions From A 3-6/6 HIA Matched Family Member Following Non-Myeloablative Allogeneic Stem Cell Transplantation. *Biol Blood and Marrow Trns* 2010 8:1107-1114. PMID 20188202
9. Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, Kwon HY, Kim J, Chute JP, **Rizzieri D**, Munchhof M, VanArsdale T, Beachy PA, Reya T. Hedgehog signaling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. 2009 458:776-779. PMID: PMC2946231
10. **Rizzieri DA**, O'Brien JA, Broadwater G, Decastro CM, Dev P, Diehl L, Beaven A, Lagoo A, Gockerman JP, Chao NJ, Moore JO. Outcomes of patients who undergo aggressive induction therapy for secondary acute myeloid leukemia. *Cancer*. 2009 115:2922-2929. PMID: 19452542
11. **Rizzieri D**, LoRusso S, Tse W, Khan K, Advani A, Moore J, Karsten V, Cahill A, Gerson SL Phase I study of temozolomide and laromustine (VNP40101M) in patients with relapsed or refractory leukemia. *Clin Lymphoma Myeloma Leuk*. 2010 Jun 1;10(3):211-6. PMID: 20511167

12. David I Marks, Tao Wang, Waleska S Perez, Joseph H Antin, Edward A Copelan, Robert Peter Gale, Biju George, Vikas Gupta, Joerg Halter, Hanna Jean Khoury, Thomas R Klumpp, Hillard M Lazarus, Victor A Lewis, Philip L McCarthy, **David A Rizzieri**, Mitchell Sabloff, Jeff Szer, Martin S Tallman, and Daniel J Weisdorf. The outcome of full intensity versus reduced intensity conditioning matched sibling or unrelated donor transplantation in adults with Philadelphia chromosome negative acute lymphoblastic leukemia in first and second complete remission. *Blood* 2010 Jul 22;116(3):366-74. PMID: PMC2913452
13. Lanasa MC, Davis PH, Datto M, Li Z, Gockerman JP, Moore JO, Decastro CM, Friedman DR, Diehl LF, Rehder C, Cook H, Daugherty FJ, Matta KM, Weinberg JB, **Rizzieri D**. Phase II Study of Cenersen, an Antisense Inhibitor of p53, in Combination with Fludarabine, Cyclophosphamide, and Rituximab for High Risk CLL. *Leuk Lymphoma*. 2012 Feb;53(2):218-24. PMID:21827374
14. Ruth Pettengell, Bertrand Coiffier, Geetha Narayanan, Fernando Hurtado de Mendoza, Raghunadharao Digumarti, Henry Gomez, Pier Luigi Zinzani, Gary Schiller, **David Rizzieri**, Giles Boland, Paul Cernohous, Lixia Wang, Christine Kuepfer, Igor Gorbachevsky, Jack W Singer Pixantrone dimaleate versus other chemotherapeutic agents as a single-agent salvage treatment in patients with relapsed or refractory aggressive non-Hodgkin lymphoma: a phase 3, multicentre, open-label, randomised trial. *Lancet Oncology*. 2012. 13:696-706. PMID 22652183
15. Faderl S, Wetzler M, **Rizzieri D**, Schiller G, Jagasia M, Stuart R, Ganguly S, Avigan D, Craig M, Collins R, Maris M, Kovacovics T, Goldberg S, Seiter K, Hari P, Greiner J, Vey N, Recher C, Ravandi F, Wang ES, Vasconcelles M, Huebner D, Kantarjian HM. Clofarabine plus cytarabine with cytarabine alone in older patients with relapsed or refractory acute myelogenous leukemia: Results from the Classic I Trial. *J. Clin Oncol*. 2012. 30:2492-2499. PMID:22585697
16. Hale GA, Shrestha S, Le-Rademacher J, Burns LJ, Gibson J, Inwards DJ, Freytes CO, Bolwell BJ, Hsu JW, Slavin S, Isola L, **Rizzieri DA**, Gale RP, Laport GG, Montoto S, Lazarus HM, Hari PN. Alternate donor hematopoietic cell transplantation (HCT) in NHL using lower intensity conditioning: a report from the CIBMTR. *Biol Blood Marrow Transplant*. 2011. 18:1036-1043. PMID:22155506
17. Jenny Zhang, Vladimir Grubor, Cassandra L Love, Anjishnu Banerjee, Kristy L Richards, Piotr Mieczkowski, Cherie H Dunphy, William WL Choi, Wing-Yan Auv, Gopesh Srivastava, Patricia L Lugar, David A **Rizzieri**, Anand S Lagoo, Leon Bernal-Mizrachi, Karen P Mann, Christopher R Flowers, Kikkeri N Naresh, Andrew M Evens, Leo I Gordon, Magdalena B Czader, Javed I Gill, Eric D Hsi, Qingquan Liu, Alice Fan, Katherine Walsh, Dereje D Jima, Micah Luftig, Ting Ni, Jun Zhu, Amy Chadburn, Shawn Levy, David B Dunson, Sandeep S Dave. The genetic landscape of immune-competent and HIV lymphoma *Infect Agent Cancer*. 2012; 7(Suppl 1): O1. Published online 2012 April 19 PMID: PMC3330083
18. Dereje D. Jima, Jenny Zhang, Cassandra Jacobs, Kristy L. Richards, Cherie H. Dunphy, William W. L. Choi, Wing Yan Au, Gopesh Srivastava, Magdalena B. Czader, David A. **Rizzieri**, Anand S. Lagoo, Patricia L. Lugar, Karen P. Mann, Christopher R. Flowers, Leon Bernal-Mizrachi, Kikkeri N. Naresh, Andrew M. Evens, Leo I. Gordon, Micah Luftig, Daphne R. Friedman, J. Brice Weinberg, Michael A. Thompson, Javed I. Gill, Qingquan Liu, Tam How, Vladimir Grubor, Yuan Gao, Ameer Patel, Han Wu, Jun Zhu, Gerard C. Blobe, Peter E. Lipsky, Amy Chadburn, Sandeep S. Dave, for the Hematologic Malignancies Research Consortium (HMRC). Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs. *Blood*. 2010. 116(23): e118–e127. PMID: PMC3012600
19. Maramattom LV, Hari PN, Burns LJ, Carreras J, Arcese W, Cairo MS, Costa LJ, Fenske TS, Lill M, Freytes CO, Gale RP, Gross TG, Hale GA, Hamadani M, Holmberg LA, Hsu JW, Inwards DJ, Lazarus HM, Marks DI, Maloney DG, Maziarz RT, Montoto S, **Rizzieri DA**, Wirk B, Gajewski JL. Autologous and Allogeneic Transplantation for Burkitt Lymphoma Outcomes and Changes in Utilization: A Report from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant*. 2012. PMID:23200705
20. Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards KL, Dunphy CH, Choi WW, Srivastava G, Lugar PL, **Rizzieri DA**, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers CR, Naresh KN, Evens AM, Chadburn A, Gordon LI, Czader MB, Gill JI, Hsi ED, Greenough A, Moffitt AB, McKinney M, Banerjee A, Grubor V, Levy S, Dunson DB, Dave SS. The genetic landscape of mutations in Burkitt lymphoma. *Nat Genet*. 2012 Nov 11;44:1321-5. PMID:2314359

D. Research Support

ACTIVE

2P01-CA047741-16A1

(Chao)

07/01/09 - 06/30/14

National Institutes of Health

Clinical trials of Haploidentical Hematopoietic Stem Cell Transplantation and Vaccine Strategies

Goal: The broad, long-term objectives and specific aims of this project are to increase hematopoietic cell donor availability and progression free and overall survival following allogeneic transplantation for malignant diseases.

3P30-CA-014236-36S2

Lyerly (PI)

09/01/2010 – 12/31/2014

National Institutes of Health

'Cancer Clinical Investigator Team Leadership Award' – to support, acknowledge, recognize outstanding clinical investigators whose participation and activities promote successful clinical research programs and to promote retention of clinical investigators in an academic career in clinical research.

Selected Industry Sponsored Clinical Trials

Duke University lists aggregated effort assigned to the following eligible industry-sponsored clinical trial projects.

CPTK787AUS39

(Rizzieri)

07/01/05 – 04/30/11

Novartis Pharmaceuticals Corporation

A Phase II Open Label Study PTK787 / ZK222584 in Adult Patients with Refractory or Relapsed Diffuse Large Cell Lymphoma. The purpose of this study is to find out if an investigational drug, PTK787 / ZK222584 is safe and effective in treating lymphoma.

Eleos, Inc.

(Rizzieri)

02/15/08 – 02/14/11

A Phase II Study of EL625 in Patients in Persistent Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. The purpose of this research study is to see if the investigational drug EL625, when combined with traditional chemotherapy (rituximab, fludarabine, and cyclophosphamide) is effective in treating chronic lymphocytic leukemia/ small lymphocytic lymphoma

STA-9090

(Rizzieri)

09/02/09 – 09/15/14

Synta Pharmaceuticals Corp.

A Phase 1/2 Study of the HSP90 Inhibitor, STA-9090, Administered Once-Weekly in Subjects with Acute Myeloid Leukemia, Acute Lymphoblastic Leukemia and Blast-phase Chronic Myelogenous Leukemia
The purpose of this study is to determine if AML, ALL, or blast-phase CML respond to an investigational drug called STA-9090.

CPX-351

(Rizzieri)

03/27/09 – 04/03/14

Celator Pharmaceuticals

Phase IIB, Multicenter, Randomized, Open-Label Trial Of CPX-351 (Cytarabine:Daunorubicin) Liposome Injection Versus Intensive Salvage Therapy In Adult Patients =60 Years Old With AML In First Relapse Following An Initial Cr >1 Month Duration. This study will help us learn more about the study drug, CPX-351, in subjects whose leukemia has come back (relapsed) after being in remission for more than 1 month.

Recently Completed Research Support

Leukemia Lymphoma Society

(Rizzieri)

07/01/09 – 06/30/11

'Scholar in Clinical Research'

Nonmyeloablative allogeneic therapy using haploidentical allogeneic transplantation. Evaluate the safety and efficacy of multiple immune modulatory strategies following non-myeloablative therapy.

CCI-779

(Rizzieri)

07/01/08 – 06/30/10

University of California – Irvine

A Phase I Study of CCI-779 in Combination with Imatinib Mesylate. The purpose of this study is to see if using CCI-779 in combination with Imatinib Mesylate is an effective treatment for refractory CML.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE		
Sandeep S. Dave	Associate Professor		
eRA COMMONS USER NAME SANDEEP.DAVE			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Northwestern University	MS	1995	Biomedical Engineering
Northwestern University Medical School	MD	1999	Medicine
Northwestern University	MBA	2000	Management

A. PERSONAL STATEMENT

I have the expertise, leadership and motivation necessary to successfully carry out the proposed work. I have a broad background in oncology and genomics, with specific training and expertise in key research areas for this application. As a postdoctoral fellow in the laboratory of Louis Staudt at the National Institutes of Health, my work was focused on the application of genomics to develop diagnostic and prognostic models in patients with lymphoma. At Duke University, I have an independent research program at Duke University focused on mechanisms underlying gene regulation in lymphomas. My first R01 grant is focused on the identification of novel therapeutic targets in patients with diffuse large B cell lymphoma. As PI or co-Investigator on several previous university- and NIH-funded grants, I laid the groundwork for the proposed research by developing broad capabilities in genomics-based research and analysis. My previous work has resulted in several high impact peer-reviewed publications including 2 first-author publications in the New England Journal of Medicine. I am thus qualified to serve as mentor for Dr. Brander's work exploring drug resistance in indolent B-cell malignancies.

Positions and Employment

1990-1991 Systems Engineer, Citibank, Bombay, India
 1991-1993 Systems Analyst Sears Financial, Chicago, IL
 1999-2001 Residency, Internal Medicine Research Track (Director: Warren Wallace), Northwestern University Medical School, Chicago, IL
 2001- 2006 Clinical Fellow, Program in Hematology-Oncology (Director: Cynthia Dunbar), NIH
 2004- 2007 Post-doctoral Fellow, Louis Staudt Laboratory, National Cancer Institute, NIH
 2007-2012 Assistant Professor, Duke University Medical Center
 2012-Present Associate Professor, Duke University Medical Center
 2012-Present Director, Molecular Genetics and Genomics Program, Duke Cancer Institute

Honors

1995-1999 Class President, Northwestern University Medical School
 1996-1998 National Chair, Committee on Community & Public Health, American Medical Student Association
 1996 Golden Stethoscope Award, Outstanding Medical Student

- 1997-1998 Medical Student Senate President
 2000, 2001 Excellence in teaching award for teaching medical students, Northwestern University Medical School
 2004-2005 Co-chair, *FELCOM*, NIH-wide organization of clinical and post-doctoral fellows
 2004 Fellows Award for Research Excellence, National Institutes of Health
 2006 NIH Federal Technology Transfer Award
 2008-Present Clinical Scientist Development Award, the Doris Duke Charitable Foundation.
 2008-Present Full Member, Duke Comprehensive Cancer Center
 2010-Present Research Scholar, American Cancer Society
 2013 Elected to American Society for Clinical Investigation, the honor society of physician-scientists

C. Selected peer-reviewed publications (from ~50).

Most relevant to the current application

1. Zhang J, Grubor V, Love CL, Banerjee A, Richards KL, Mieczkowski PA, Dunphy C, Choi W, Au WY, Srivastava G, Lugar PL, Rizzieri DA, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers C, Naresh K, Evens A, Gordon LI, Czader M, Gill JI, Hsi ED, Liu Q, Fan A, Walsh K, Jima D, Smith LL, Johnson AJ, Byrd JC, Luftig MA, Ni T, Zhu J, Chadburn A, Levy S, Dunson D, **Dave Sandeep S (Corresponding Author)** Genetic heterogeneity of diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A*. 2013 (epub).
2. Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards KL, Dunphy CH, Choi WW, Srivastava G, Lugar PL, Rizzieri DA, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers CR, Naresh KN, Evens AM, Chadburn A, Gordon LI, Czader MB, Gill JI, Hsi ED, Greenough A, Moffitt AB, McKinney M, Banerjee A, Grubor V, Levy S, Dunson DB, **Dave Sandeep S (Corresponding Author)** The genetic landscape of mutations in Burkitt lymphoma. *Nature Genetics*. 2012 Nov 11;44(12):1321-5.
3. Walsh K, McKinney M, Love C, Liu Q, Fan A, Patel A, Smith J, Beaven A, Jima D, **Dave Sandeep S (Corresponding Author)**. PAK1 Mediates Resistance to PI3 Kinase Inhibition in Lymphomas. *Clinical Cancer Research* 2013 (epub).
4. Jima DD, Zhang J, Jacobs C, Richards KL, Dunphy CH, Choi WL, Au WY, Srivastava G, Czader MG, Rizzieri DA, Lagoo AS, Lugar PL, Mann KP, Flowers CR, Bernal-Mizrachi L, Sunay S, Naresh KN, Evens AM, Gordon LI, Luftig M, Friedman DR, Weinberg JB, Thompson MA, Gill JI, Kahl BS, Gao Y, Xie B, Patel A, Wu H, Zhu J, Dressman H, Lipsky PE, Chadburn A, **Dave Sandeep S (Corresponding Author)** Deep sequencing of the small RNA transcriptome of normal and malignant human B cells identifies hundreds of novel microRNAs. *Blood*. 2010;116(23):e118-27
5. Zhang J, Jima DD, Jacobs C, Fischer R, Gottwein E, Huang G, Lugar PL, Lagoo AS, Rizzieri DA, Friedman DR, Weinberg JB, Lipsky PE, **Dave Sandeep S (Corresponding Author)** Patterns of microRNA expression characterize stages of human B cell differentiation. *Blood*. 2009 113(19):4586-94

Additional recent publications of importance to the field (in chronological order)

1. Iqbal J, Sanger WG, Horsman DE, Rosenwald A, Pickering DL, Dave B, **Dave Sandeep** et al. BCL2 translocation defines a unique tumor subset within the germinal center B cell-like diffuse large B-cell lymphoma. *Am J Pathol*. 2004 Jul;165(1):159.
2. Fu, K, Weisenburger DD, Greiner T, **Dave Sandeep S**, et al. Cyclin D1-negative Mantle Cell Lymphoma: A Clinicopathological Study Based on Gene Expression Profiling, *Blood* 2005; 106(13):4315
3. Ngo VN, Davis RE, Lamy L, Yu X, Lenz G, Lam L, **Dave Sandeep S**, et al. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* 2006;441(7089):106
4. Lenz G, Nagel I, Siebert R, Roschke AV, Sanger W, Wright GW, **Dave Sandeep S** et al, Aberrant immunoglobulin class switch recombination and switch translocations in activated B cell-like diffuse large B cell lymphoma. *J Exp Med* 2007;204(3):633
5. Cuadros M, **Dave Sandeep S**, Jaffe ES, et al. Identification of a proliferation signature related to survival in nodal peripheral T-cell lymphomas. *J Clin Oncol* 2007; 25(22):3321
6. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave Sandeep S, et al Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci U S A*. 2008;105(36):13520
7. Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, **Sandeep S**, et al. IRF4 addiction

in multiple myeloma, *Nature*. 2008 454(7201):226-31

8. Lenz G, Wright G, **Dave Sandeep S**, et al Stromal Gene Signatures in Large-B-Cell Lymphomas. *N Engl J Med*. 2008;359(22):2313
9. Dunleavy K, Pittaluga S, Czuczman MS, **Dave Sandeep S**, et al Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B cell lymphoma. *Blood*. 2009 [Epub ahead of print] PMID: 19202128
10. Longo NS, Lugar PL, Yavuz S, Zhang W, Krijger PH, Russ DE, Jima DD, **Dave Sandeep S**, et al. Analysis of somatic hypermutation in X-HlgM syndrome reveals specific deficiencies in mutational targeting, *Blood*. 2009 113(16):3706-15

D. Selected Research Support

Ongoing Research Support

National Institutes of Health R01CA136895-02; Sandeep Dave (PI) 12/1/08 -11/30/13

Molecular targets in diffuse large B cell lymphoma

The aim of this project is to identify new therapeutic targets in diffuse large B cell lymphoma.

Role: PI

American Cancer Society; Sandeep Dave (PI) 7/1/10 - 6/30/14

MicroRNAs in Aggressive Lymphoma

The aim of this project is to identify a role for microRNA in aggressive lymphoma.

Role: PI

Completed Support

National Institutes of Health 1R21-CA156168; (6/1/11-5/31/13)

Exome sequencing in lymphomas

Role:PI

National Institutes of Health K12-CA-100639 7/1/07-12/1/08

Role of MicroRNAs in Normal and B cells.

The goal of this project is to identify microRNAs that characterize normal mature B cells.

Role: Project PI

AIDS Malignancy Consortium Development Grant 7/1/08-7/1/09

Molecular phenotypes of AIDS Lymphoma

The goal of this project is to identify microRNAs that characterize normal mature B cells.

Role: Co-PI with Amy Chadburn

Doris Duke Charitable Foundation; Sandeep Dave (PI) 8/1/08 - 7/31/11

Role of NF-KB in Lymphoma

The aim of this project is to identify a role for NF-KB in aggressive lymphomas.

Role: PI