FORM 8-K

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PHARMACYCLICS INC

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UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT
Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934

Date of Report (Date of earliest event reported): **November 7, 2011**

PHARMACYLCICS, INC.

(Exact name of registrant as specified in its charter)

Delaware	000-26658	94-3148201
(State or other jurisdiction of incorporation)	(Commission File Number)	(IRS Employer Identification No.)
995 E. Arques Avenue, Sunnyvale, California		94085-4521
(Address of principal executive offices)		(Zip Code)

Registrant's telephone number, including area code: (408) 774-0330

(Former name or former address, if changed since last report.)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions (*see* General Instruction A.2. below):

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)

☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)

☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))

☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Item 7.01. Regulation FD Disclosure.

Pharmacyclics, Inc. submitted eight abstracts to the American Society of Hematology ("***ASH***") which summarize data on PCI-32765 to be presented at their annual meeting in San Diego which starts December 10, 2011. On November 7, 2011, the ASH released such abstracts to the public by posting them on its website. The eight abstracts are:

- The Bruton's Tyrosine Kinase Inhibitor PCI-32765 Is Highly Active As Single-Agent Therapy in Previously-Treated Mantle Cell Lymphoma (MCL): Preliminary Results of a Phase II Trial
- The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Induces Durable Responses in Relapsed or Refractory (R/R) Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): Follow-up of a Phase Ib/II Study
- The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Modulates Chronic Active BCR Signaling and Induces Tumor Regression in Relapsed/Refractory ABC DLBCL
- Targeting Bruton's Tyrosine Kinase with PCI-32765 Blocks Growth and Survival of Multiple Myeloma and Waldenström Macroglobulinemia via Potent Inhibition of Osteoclastogenesis, Cytokines/Chemokine secretion, and Myeloma Stem-like Cells in the Bone Marrow Microenvironment
- The Bruton Tyrosine Kinase Inhibitor, PCI-32765, Inhibits Activation and Proliferation of Human Chronic Lymphocytic Leukemia Cells in the NSG Xenograph Mouse Model of the Tissue Microenvironment
- Btk Inhibitor, PCI-32765, Delays CLL Progression in a TCL1 Adoptive Transfer Model by Impairing Migration and Cell Proliferation
- Egress of CD19+CD5+ Cells Into Peripheral Blood Following Treatment with the Bruton Tyrosine Kinase Inhibitor, PCI-32765, in Mantle Cell Lymphoma Patients
- Activity of Bruton's Tyrosine Kinase (Btk) Inhibitor PCI-32765 in Mantle Cell Lymphoma (MCL) Identifies Btk As a Novel Therapeutic Target

The full text of the abstracts are attached to this Current Report on Form 8-K as Exhibits 99.1 , 99.2 , 99.3, 99.4, 99.5, 99.6, 99.7, and 99.8 incorporated herein by reference.

The information in Item 7.01 of this Form 8-K, and the related exhibits, shall not be deemed "filed" for purposes of Section 18 of the Securities Exchange Act of 1934 (the " ***Exchange Act*** ") or otherwise subject to the liabilities of that section, nor shall it be deemed incorporated by reference in any filing under the Securities Act of 1933, as amended, or the Exchange Act, except as expressly set forth by specific reference in such a filing.

Item 9.01. Financial Statements and Exhibits.

(d) Exhibits

99.1	Abstract - The Bruton's Tyrosine Kinase Inhibitor PCI-32765 Is Highly Active As Single-Agent Therapy in Previously-Treated Mantle Cell Lymphoma (MCL): Preliminary Results of a Phase II Trial
99.2	Abstract - The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Induces Durable Responses in Relapsed or Refractory (R/R) Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): Follow-up of a Phase Ib/II Study
99.3	Abstract - The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Modulates Chronic Active BCR Signaling and Induces Tumor Regression in Relapsed/Refractory ABC DLBCL
99.4	Abstract - Targeting Bruton's Tyrosine Kinase with PCI-32765 Blocks Growth and Survival of Multiple Myeloma and Waldenström Macroglobulinemia via Potent Inhibition of Osteoclastogenesis, Cytokines/Chemokine secretion, and Myeloma Stem-like Cells in the Bone Marrow Microenvironment
99.5	Abstract - The Bruton Tyrosine Kinase Inhibitor, PCI-32765, Inhibits Activation and Proliferation of Human Chronic Lymphocytic Leukemia Cells in the NSG Xenograph Mouse Model of the Tissue Microenvironment

99.6	Abstract - Btk Inhibitor, PCI-32765, Delays CLL Progression in a TCL1 Adoptive Transfer Model by Impairing Migration and Cell Proliferation
99.7	Abstract - Egress of CD19+CD5+ Cells Into Peripheral Blood Following Treatment with the Bruton Tyrosine Kinase Inhibitor, PCI-32765, in Mantle Cell Lymphoma Patients
99.8	Abstract - Activity of Bruton's Tyrosine Kinase (Btk) Inhibitor PCI-32765 in Mantle Cell Lymphoma (MCL) Identifies Btk As a Novel Therapeutic Target

SIGNATURES

Pursuant to the requirements of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

Date: November 7, 2011

By: /s/ Rainer M. Erdtmann
Name: Rainer M. Erdtmann
Title: Vice President, Finance & Administration and Secretary

Exhibit Index

Exhibit Number	Description of Exhibit
99.1	Abstract - The Bruton's Tyrosine Kinase Inhibitor PCI-32765 Is Highly Active As Single-Agent Therapy in Previously-Treated Mantle Cell Lymphoma (MCL): Preliminary Results of a Phase II Trial
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Title: The Bruton's Tyrosine Kinase Inhibitor PCI-32765 Is Highly Active As Single-Agent Therapy in Previously-Treated Mantle Cell Lymphoma (MCL): Preliminary Results of a Phase II Trial (ASH 2011 Annual Meeting Abstract #442)

Luhua Wang, MD[1], Peter Martin, MD[2], Kristie A. Blum, MD[3], Brad S. Kahl, MD[4], Lauren S. Maeda, MD[5], Ranjana Advani, MD[6], Michael E. Williams, MD[7], Simon Rule, MD[8*], Sara Rodriguez[9*], Ching-Fai Pang, PhD[9*], Eric Hedrick, MD[9] and Andre Goy, MD[10]

[1]Lymphoma/Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX; [2]Division of Hematology-Oncology, Weill Cornell Medical College, New York, NY; [3]The Ohio State University, Columbus, OH; [4]Department of Medicine-Hematology/Oncology, University of Wisconsin, Madison, WI;[5]Department of Medicine, Division of Oncology, Stanford University Medical Center, Stanford, CA; [6]Med/Oncology, Stanford University Medical Center, Stanford, CA; [7]University of Virginia, Charlottesville, VA; [8]Department of Haematology, Derriford Hospital, Plymouth, United Kingdom; [9]Pharmacyclics, Sunnyvale, CA; [10]John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, NJ

Introduction: Bruton's tyrosine kinase (Btk) is a central mediator of B-cell receptor signaling which is essential for normal B-cell development. PCI-32765 is an orally administered irreversible inhibitor of Btk that induces apoptosis and inhibits cellular migration and adhesion in malignant B-cells. In a phase I trial of PCI-32765 in relapsed B-cell malignancies, objective responses were observed in seven of nine patients with MCL. Reported here are preliminary results of an ongoing phase II study of single-agent PCI-32765 in previously treated MCL.

Methods and Patients: Patients with relapsed or refractory MCL who were either bortezomib-naïve or bortezomib-exposed (prior treatment with at least 2 cycles of bortezomib) were eligible for study PCYC-1104. PCI-32765 was administered orally at 560mg daily (in continuous 28-day cycles) until disease progression. Bortezomib-naive and bortezomib-exposed cohorts were evaluated separately. Tumor response was evaluated every 2 cycles and classified by 2007 NHL IWG criteria.

Results: A total of 48 patients (29 bortezomib-naive, 19 bortezomib-exposed) have been enrolled on study PCYC-1104 between February 16, 2011 and July 20, 2011. The median age is 67 years (62-72). The median number of prior treatment regimens is 2 (1-5). Five patients (13%) had received prior autologous or allogeneic stem cell transplantation. Seven patients (15%) had bulky disease. Thirty-nine patients who have initiated treatment and have reported adverse event (AE) information are the subject of this preliminary report. Twenty-four patients (12 bortezomib-naive, 12 bortezomib-exposed) have undergone at least 1 follow-up tumor assessment and are evaluable for efficacy. Treatment has been well tolerated. No patients have discontinued treatment due to AEs. Grade 1 or 2 diarrhea, fatigue, and nausea have been the most frequently reported AEs. Grade >3 AEs considered potentially related to PCI-32765 have occurred in 4/39 patients (11%). Serious AEs (SAEs) have occurred in 8/39 patients (21%); 2 SAEs (1 rash, 1 febrile neutropenia) were considered potentially related to PCI-32765. One death, in a patient who was enrolled but did not receive PCI-32765 due to rapid disease progression, has occurred on study. The objective response rate (ORR) by IWG criteria is 67% (16/24); ORR is 58% (7/12) in the bortezomib-naive cohort and 75% (9/12) in the bortezomib-exposed cohort. To date, 35/39 patients remain on PCI-32765; reasons for discontinuation include progressive disease (n=3) and investigator decision (n=1).

Conclusions: Preliminary data from a phase II trial suggests that the potent Btk inhibitor PCI-32765 is well tolerated and induces a high rate of objective responses in patients with relapsed or refractory MCL. More mature safety and efficacy data will be updated in the presentation. Phase III trials of PCI-32765 in MCL are planned.

Title: The Bruton's Tyrosine Kinase (BTK) Inhibitor PCI-32765 Induces Durable Responses in Relapsed or Refractory (R/R) Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL): Follow-up of a Phase Ib/II Study (ASH 2011 Annual Meeting Abstract #983)

Susan O'Brien, MD[1], Jan A. Burger, MD, PhD[2], Kristie A. Blum, MD[3], Richard R. Furman, MD[4], Steven E. Coutre, MD[5], Jeff Sharman, MD[6*], Ian W. Flinn, MD, PhD[7], Barbara Grant, MD[8*], Nyla A. Heerema, PhD[9], Amy J. Johnson, PhD[3], Tasheda Navarro[10*], Eric Holmgren, PhD[10*], Eric Hedrick, MD[10] and John C. Byrd, MD[11]

[1]Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX; [2]Department of Leukemia, The University of Texas, M.D. Anderson Cancer Center, Houston, TX; [3]The Ohio State University, Columbus, OH; [4]Department of Medicine, Division of Hematology-Oncology, Weill Cornell Medical College, New York, NY; [5]Divisions of Hematology and Oncology and Stanford Cancer Center, Stanford University School of Medicine, Stanford, CA; [6]US Oncology, Springfield, OR; [7]Sarah Cannon Research Institute, Nashville, TN; [8]Medicine, Vermont Cancer Center, University of Vermont, Burlington, VT; [9]Pathology, The Ohio State University, Columbus, OH; [10]Pharmacyclics, Inc, Sunnyvale, CA; [11]Division of Hematology, The Ohio State University, Columbus, OH

Introduction: Btk is a central mediator of B-cell receptor signaling which is essential for normal B-cell development. PCI-32765 is an orally-administered irreversible inhibitor of Btk which induces apoptosis and inhibits cellular migration and adhesion in malignant B-cells. An early analysis of the phase Ib/II study PCYC-1102 showed PCI-32765 to be highly active and tolerable in patients with CLL (Byrd, ASCO 2011). Here we report longer-term follow-up of this multicenter phase Ib/II trial.

Methods and Patients: Two cohorts of CLL patients (previously untreated ≥65 years old and relapsed/refractory [R/R] disease following at least 2 prior therapies, including fludarabine) were treated with oral PCI-32765 administered daily for 28-day cycles until progression of disease. Doses of 420mg (previously untreated and R/R) and 840mg daily (R/R) were examined. The patients with R/R disease are the subject of this report.

Results: Sixty-one R/R CLL/SLL patients were enrolled (420mg cohort n=27, 840mg cohort n=34). The median follow-up time for the 420mg cohort is 10.2 months and for the 840mg cohort is 6.5 months. The median number of prior treatment regimens for the 420mg cohort was 3 (2-10) and for the 840mg cohort was 5 (1-12). Seventy-two percent of patients had at least one poor-risk molecular feature: del(17p) 31%, del(11q) 33%, IgV_H un-mutated 57%. Treatment has been well tolerated. Two patients have discontinued for adverse events (AE); 6 patients have required reduction of PCI-32765 dose (420mg cohort 2/27, 840mg cohort 4/34). Grade 1 or 2 diarrhea, fatigue, nausea, and ecchymosis have been the most frequently reported AEs. Serious AEs (SAEs) have occurred in 38% of patients; SAEs considered potentially related to PCI-32765 have occurred in 10% of patients. Grade ≥3 AEs considered potentially related to PCI-32765 occurred in 21% of patients. A characteristic pattern of response, with a transient phase of lymphocytosis typically peaking within the first 2 months of Rx, followed by resolution over time, has been observed in the majority of patients. Objective response (ORR; PR + CR) by IWCLL criteria in the 420mg cohort, previously reported as 48% with 6.2 months median follow-up (Byrd, et al ASCO 2011), is now 70% with 10.2 months median follow-up. ORR in the 840mg cohort is 44% at 6.5 months median follow-up. An additional 19%, and 35% of patients in these cohorts, respectively, have a nodal PR (>50% reduction in aggregate lymph node size) with residual lymphocytosis. ORR appears to be independent of molecular risk features. Eighty-two percent of patients (50/61; 420mg cohort 22/27, 840mg cohort 28/34) remain on PCI-32765. Only 8% (5/61) of patients have had progressive disease (PD); 6-month PFS is 92% in the 420mg cohort and 90% in the 840mg cohort. Treatment cessation not related to PD or AE includes: death (n=2) or investigator discretion (n=3).

Conclusions: The potent Btk inhibitor PCI-32765 is well tolerated and is associated with high rates of 6-month PFS in R/R CLL/ SLL. Phase III trials of PCI-32765 in CLL/ SLL are planned.

Title: The Bruton's Tyrosine Kinase (Btk) Inhibitor PCI-32765 Modulates Chronic Active BCR Signaling and Induces Tumor Regression in Relapsed/Refractory ABC DLBCL (ASH 2011 Annual Meeting Abstract #2716)

Louis M. Staudt[1], Kieron Dunleavy[1], **Joseph Buggy**[2], Eric Hedrick[2], Nicole Lucas[1], Stefania Pittaluga[1], Sameer Jhavar[1], Roland Schmitz[1], Mickey Williams[1], Jason Lih[1], Elaine S. Jaffe[1], and Wyndham H. Wilson[1]

[1]National Cancer Institute, Bethesda, MD; [2]Pharmacyclics, Inc., Sunnyvale, CA

Background: Btk is a tyrosine kinase involved in B cell receptor (BCR) signal transduction. Recent studies indicate that chronic active BCR signaling is a pathogenic mechanism in ABC DLBCL and this chronic activation engages the classic NF-κB pathway. Mutations in the BCR pathway (CD79 A/B and CARD11) and toll like receptor (TLR) pathway (MYD88) lead to constitutive NF-κB activation in ABC DLBCL. PCI- 32765 kills ABC DLBCL cell lines with constitutive BCR signaling but has no effect on ABC and GBC DLBCL cell lines that do not rely on constitutive BCR signaling. PCI- 32765 is an oral, well-tolerated and irreversible inhibitor of Btk.

Study Design: Patients with relapsed/refractory ABC DLBCL received PCI-32765 at a fixed dose of 560 mg po once daily x 35 days (1 cycle). Patients underwent CT and FDG-PET scanning pretreatment and every 2 cycles. Where possible, pre-treatment and 48-hour post-treatment tumor biopsies were performed for gene expression profiling (GEP) and mutational analysis of CD79A/B, CARD11 and MYD88.

Results: Eight of 15 planned patients are enrolled. Characteristics include median (range) age 54 (40-79); LDH > normal limits (63%); any extranodal site (75%) and stage 4 disease (63%). IPI distribution was 0-2 (25%) and 3-5 (75%). Patients received a median (range) of 3 (1-6) prior chemotherapy regimens. Best response by IWG criteria include CR: 2 (25%) for 11+ and 5 months; SD (stable disease) 3 (37%) for 4, 2 and 2 months; and PD (progressive disease) 3 (38%). One patient with who achieved SD was primary refractory and achieved a 25% tumor reduction with PCI-32765 and currently is in CR following allogeneic BMT. PCI-32765 was well-tolerated without significant side effects. Toxicities that are possibly related to PCI-32765 include diarrhea (grade 1) in 2 patients, nausea (grade 1) in 2 patient and fatigue (grades 1-2) in 4 patients. 4CD79B mutations were uncovered in two patients, the SD who achieved a 25% tumor response and one who achieved CR. Of note, the other patient who achieved CR did not have the CD79B mutation, suggesting that chronic active BCR signaling may occur in the absence of this mutation. None of the patients had MYD88 or CARD11 mutations. Comparison of the pre-treatment and on-treatment biopsy samples by gene expression profiling was completed on 6 patients (1 CR and 5 SD/PD). Gene expression signatures reflecting tumor infiltrating immune cells, including CD8+ T cells and macrophages, were diminished by PCI-32765 treatment in one patient in CR and the one patient in SD who achieved a 25% tumor reduction, but not in the others. One hypothesis to explain this observation would be cytokine modulation since chronic active BCR signaling activates the NF- κB pathway, thereby promoting secretion of IL-6, IL-10 and multiple chemokines. Interestingly, PCI-32765 has been reported to down regulate cytokines including IL-6 in effector cells in a murine model of immunecomplex disease (Chang et al. Arthritis Research and Therapy. In Press). In these same two cases, PCI-32765 treatment lowered the expression of a gene expression signature of type I interferon responses, suggesting a crosstalk between chronic active BCR signaling and the TLR/MYD88 pathway, which promotes type I interferon signaling in ABC DLBCL.

Conclusions: The Btk inhibitor PCI-32765 has clinical activity in relapsed/refractory ABC DLBCL and modulates chronic active BCR signaling in responders. Thus, chronic active BCR signaling is a tractable therapeutic target in ABC DLBCL.

Title: Targeting Bruton's Tyrosine Kinase with PCI-32765 Blocks Growth and Survival of Multiple Myeloma and Waldenström Macroglobulinemia via Potent Inhibition of Osteoclastogenesis, Cytokines/Chemokine secretion, and Myeloma Stem-like Cells in the Bone Marrow Microenvironment (ASH 2011 Annual Meeting Abstract #883)

Yu-Tzu Tai,[1] Betty Chang,[2] Sun-Young Kong,[1] Mariateresa Fulciniti,[1] Guang Yang,[3] Yolanda Calle,[4] Yiguo Hu,[1] Jianhong Lin,[1] Jian-Jun Zhao,[1] Antonia Cagnetta,[1] Michele Cea,[1] Michael A Sellitto,[1] Michelle Chen,[1] Daniel R Carrasco,[1] Joseph J. Buggy,[2] Laurence Elias,[2] Zachary R. Hunter,[3] Steven P. Treon,[3] William Matsui,[5] Paul Richardson,[1] Nikhil C. Munshi,[1] Kenneth C Anderson[1]

[1]LeBow Institute for Myeloma Therapeutics and Jerome Lipper Center for Multiple Myeloma Research, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; [2]Pharmacyclics, Inc., Sunnyvale, CA, USA; [3]Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA; [4]Dept. of Haematological Med., King's College London, London, UK; [5]Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Specific expression of Bruton's tyrosine kinase (Btk) in osteoclasts (OC), but not osteoblasts (OB), suggests its role in regulating osteoclastogenesis. Although Btk is critical in B cell maturation and myeloid function, it has not been characterized in plasma cell malignancies including multiple myeloma (MM) and Waldenström Macroglobulinemia (WM). In the present study, we investigate effects of PCI-32765, an oral, potent, and selective Btk inhibitor with promising clinical activity in B-cell malignancies, on OC differentiation and function within MM bone marrow (BM) microenvironment, as well as on MM and WM cancer cells. We further define molecular targets of Btk signaling cascade in OCs and MM in the BM milieu. In CD14+ OC precursor cells, RANKL and M-CSF stimulated phosphorylation of Btk in a time-dependent fashion; conversely, PCI-32765 abrogated RANKL/M-CSF-induced activation of Btk and downstream PLCγ2. Importantly, PCI-32765 decreased number of multinucleated OC (>3 nuclei) by tartrate-resistant acid phosphatase (TRAP) staining and inhibited the secretion of TRAP5b (ED_{50} = 17 nM), a specific mature OC marker. It disrupted OC formation with significantly reduced bone resorption activity, as evidenced by diminished pit formation on dentine slices. Moreover, lack of effect of Dexamethasone on OC activity was overcome by combination of Dexamethasone with PCI-32765. PCI-32765 significantly reduces cytokine and chemokine secretion from OC cultures, including MIP1α, MIP1β, IL-8, TGFβ1, RANTES, APRIL, SDF-1, and activin A (ED_{50} = 0.1-0.48 nM). It potently downregulated IL-6, SDF-1, MIP1α, MIP1β, and M-CSF in CD138-negative cell cultures from active MM patients, associated with decreased TRAP staining in a dose-dependent manner. In MM and WM cells, immunoblotting analysis confirmed a higher Btk expression in CD138+ cells from majority of MM patients (4 out of 5 samples) than MM cell lines (5 out of 9 cell lines), whereas microarray analysis show an enhanced expression of Btk and its downstream signaling components in WM cells than in CD19+ normal bone marrow cells. Importantly, SDF-1 activated Btk and its downstream pPLCγ2, pAKT, and pERK in 4 MM cell lines, which is specifically blocked by PCI-32765. PCI-32765 further blocked SDF-1-induced adhesion and migration of MM cell lines and patient MM cells. It decreased cytokine expression (MIP1α, MIP1β) at mRNA level in MM and WM tumor cells, correlated with inhibition of Btk-mediated pPLCγ2, pERK and NF-κB activation. Significantly, PCI-32765 inhibits MM cell growth and survival triggered by IL-6 and coculture with BM stromal cells (BMSCs) or OCs. Furthermore, myeloma stem-like cells express Btk and PCI-32765 (10-100 nM) specifically blocks their abilities to form colonies from MM patients (n=5). In contrast, PCI-32765 has no effects on Btk-negative BMSCs and OBs, and does not affect Btk-expressing dendritic cells. Finally, oral administration of PCI-32765 (12 mg/kg) in mice significantly suppressed MM cell growth ($p<0.03$) and MM cell-induced osteolysis on implanted human bone chips in a humanized myeloma (SCID-hu) model. Together, these results provide compelling evidence to target Btk in the BM microenvironment against MM and WM, strongly supporting clinical trials of PCI-32765 to improve patient outcome in MM and WM.

Title: The Bruton Tyrosine Kinase Inhibitor, PCI-32765, Inhibits Activation and Proliferation of Human Chronic Lymphocytic Leukemia Cells in the NSG Xenograph Mouse Model of the Tissue Microenvironment (ASH 2011 Annual Meeting Abstract #596)

Sarah E. M. Herman[1], Xiameng Sun[1], Joseph Buggy[2], Georg Aue[1], Patricia Pérez-Galán[1,3], and Adrian Wiestner[1]

[1]Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland; [2]Pharmacyclics, Sunnyvale, California; [3]Department of Hemato-Oncology, IDIBAPS, Barcelona, Spain

PCI-32765, a specific inhibitor of Bruton's tyrosine kinase (Btk), can disrupt several signaling pathways involved in tumor microenvironment interactions. *In vitro*, PCI-32765 has been demonstrated to induce apoptosis, to varying degrees, in tumor cells and prevent CpG-ODN induced proliferation of cultured chronic lymphocytic leukemia (CLL) cells (Herman et al, Blood 2011). PCI-32765 has been shown to be well tolerated in CLL with preliminary clinical trial data showing that >85% (34/39) of patients remained on therapy at a median follow-up of four months. In addition, a significant shrinkage of lymph nodes has been observed in the majority of patients displaying lymphadenopathy. As with other B-cell receptor (BCR) directed therapies, PCI-32765 results in an initial increase in the absolute lymphocyte count. These observations are not explainable by the available *in vitro* data, demonstrating the need for *in vivo* investigation. In order to study the effect of PCI-32765 *in vivo* we chose to use the recently established NOD SCID gamma null (NSG) - human CLL xenograft model with some modifications (Bagnara et al., Blood 2011). NSG mice were conditioned with 25 mg/kg busulfan 24 hours before injection of 1 x 10^8 CLL peripheral blood mononuclear cells previously labeled with 1μM CFSE. We first demonstrated that xenografted CLL cells isolated from the mouse spleen acquire an activated phenotype and proliferate, mimicking the phenotype of CLL cells isolated from human lymph nodes (Sun et al., abstract submitted). Next we sought to use this model to investigate the effect of PCI-32765 on CLL cell activation and proliferation. Mice received PCI-32765 or vehicle in their drinking water at 0.16 mg/ml dissolved in 1% HP-beta-CD starting at the time of busulfan treatment. Mice were bled weekly and sacrificed between 3 and 4 weeks post xenografting. We found that PCI-32765 treatment resulted in a significant reduction in proliferation (defined as CFSE low cells) compared to mice that received vehicle water; this was observed in all three biological compartments: peripheral blood (84.5% decrease, p=0.007), spleen (72.4% decrease, p=0.012) and bone marrow (92.5% decrease, p=0.049). In comparison, PCI-32765 treatment did not result in a significant reduction in T-cell proliferation in any of the compartments (p>0.4). Although peripheral blood CLL counts were comparable between treated and untreated mice, we found that there were substantially more CLL cells in the spleens of the vehicle treated mice than in those of the PCI-32765 treated mice. In contrast, no differences in T-cell number or localization were observed between treated and untreated mice. Lastly, we sought to determine whether activation of CLL cells in the microenvironment could be blocked by PCI-32765. As we have previously shown, CLL cells in the human lymph node display a gene signature indicating B-cell receptor (BCR) and NF- κB activation compared to CLL cells in the peripheral blood (Herishanu et al., Blood 2011). We used quantitative RT-PCR (pre-designed Taqman Gene Expression assays) to measure expression of representative BCR and NF- κB target genes. PCI-32765 significantly reduced expression of EGR1 (p=0.049), EGR3 (p=0.023) and GFI1 (p=0.023) (BCR signature) and CCL3 (p=0.013) and CCND2 (p=0.046) (NF- κB signature) compared to vehicle treated mice. In addition, we also observed decreases in the proliferation gene signature (CDT1, PCNA and RRM2) (signature score, p=0.035) in the CLL cells from mice treated with PCI-32765; consistent with the assessed CFSE proliferation measurements. Taken together, our results show that PCI-32765 inhibits CLL activation and proliferation in the tissue microenvironment *in vivo* without affecting T-cell proliferation. These results demonstrate that targeting Btk is sufficient to block key interactions between tumor cells and the microenvironment and thus warrants the use of PCI-32765 as a targeted agent in CLL.

This work was supported by the Intramural Research Program of the National, Heart, Lung and Blood Institute

Title: BtK inhibitor, PCI-32765, delays CLL progression in a TCL1 adoptive transfer model by impairing migration and cell proliferation (ASH 2011 Annual Meeting Abstract #982)

Shih-Shih Chen[1], Joseph Buggy[2], Jan A. Burger[3], and Nicholas Chiorazzi[1]

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Bruton's tyrosine kinase (Btk) is critical for B-lymphocyte function, due to its involvement in key functions in B-cells, e.g., maturation, activation, and trafficking. These functions are mediated by several receptors, including the B-cell antigen receptor (BCR) and the chemokine receptor CXCR4. Both BCR and CXCR4 play important roles in chronic lymphocytic leukemia (CLL), providing leukemic cells with survival and proliferation advantages. Initial studies in CLL suggest that inhibiting Btk with PCI-32765 (Pharmacyclics, Inc.) is an effective therapeutic, reducing the size of solid lymphoid tissues leading initially to lymphocytosis and eventually to decreased blood absolute lymphocyte counts (ALCs).

To understand the effect of blocking Btk-mediated signaling in CLL, we utilized an accelerated adoptive transfer CLL mouse model, injecting $5X10^6$ TCL1 leukemia cells into SCID mice that succumb 5-6 weeks after cell transfer. Total 45 mice were injected, separated into 3 groups, and then treated with PCI-32765, at either 2, 3 or 4 weeks after cell transfer, with 5 mice from each group receiving either vehicle control or PCI-32765 (5 or 25 mg/kg/day) in daily drinking water. Mice were bled to track changes in ALCs.

Animals treated at 2-weeks post cell transfer with the suboptimal (5mg/kg/day) and optimal (25mg/kg/day) doses exhibited a transient lymphocytosis at day 4, with a 7- and 10-fold increase in circulating TCL1 leukemia cells, respectively. By day 7, these levels had fallen to those of untreated mice. Until week 6, mice receiving the optimal dose of PCI-32765 at weeks 2 and 3 but not week 4, appeared healthy and had significantly reduced ALCs. These mice had small or no lymph nodes (LNs) and significantly smaller livers and spleens with markedly reduced leukemic infiltration. In contrast, mice receiving vehicle control or sub-optimal dose of PCI-32765 exhibited lethargy, weight loss, and hunched posture; these animals had massive lymphocytosis, huge hepatosplenomegaly, and lymphadenopathy.

Surprisingly, the delayed disease progression by PCI-32765 correlates with blocked CXCR4 surface membrane recycling in CLL cells. In addition, there were significantly repressed levels of phosphorylated phospholipase C-gamma 2 (PLCγ2) in optimally treated mice. This is relevant because phosphorylation of PLCγ2 by BTK influences chemokine-controlled cell migration, at least partially through levels of CXCL12 or its receptor CXCR4. *In vivo* studies of CLL patients suggest that peripheral blood cells bearing a $CXCR4^{BR}CD5^{DIM}$ ($R4^{BR}$) surface phenotype are more likely to re-enter lymphoid tissues, while cells expressing $CXCR4^{DIM}CD5^{BR}$ ($R4^{dim}$) have more likely recently left solid tissues. Here, we found spleen cells in optimally treated mice had significantly lower percentages of $R4^{BR}$; while in the blood, there was an increased population of $R4^{DIM}$ cells. These data suggest that the smaller spleen and LN sizes of treated mice are due to promoted migration of CLL cells out of lymphoid tissues and blocked return from blood.

In vivo effect of PCI-32765 on TCL1 cell proliferation was also evaluated. BrdU was injected in mice 24 hours prior to sacrifice. As TCL1 cells proliferate in lymphoid organs, significantly repressed BrdU incorporation was demonstrated in spleen and LN cells of optimally treated mice, consistent with PCI-32765 inhibiting proliferation. In addition, in support of enhanced migration of cells out of lymphoid tissues, there was a ~3-4 fold increase in BrdU-labeled cells in blood after optimal PCI-32765 treatment.

Finally, the effects of PCI-32765 on TCL1 cell homing was assessed by injecting SCID mice with $2.5x10^6$ $R4^{DIM}$ blood cells from either controls or optimally treated mice. 24 hours later, mice engrafted with $R4^{DIM}$ cells from PCI-32765 treated animals had ~30-50 fold fewer leukemic cells in spleen ($p=0.018$); and ~1.5 fold increased CLL cells in blood ($p=0.021$), further supporting blocked homing by PCI-32765.

Collectively, our data suggest that targeting Btk, its receptors, and the downstream targets that require its use delays CLL progression. This effect is, at least partially, due to repressed surface CXCR4 expression and blocked cell proliferation, mediated either directly in CLL cells or indirectly, by minimizing the likelihood of receiving trophic stimuli via the BCR or CXCR4 in a solid tissue microenvironment.

Title: Egress of $CD19^+CD5^+$ Cells Into Peripheral Blood Following Treatment with the Bruton Tyrosine Kinase Inhibitor, PCI-32765, in Mantle Cell Lymphoma Patients (ASH 2011 Annual Meeting Abstract #954)

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PCI-32765 is an orally administered, highly potent and specific inhibitor of Bruton tyrosine kinase (BTK) in clinical development for the treatment of B cell lymphoproliferative diseases. Patients with chronic lymphocytic leukemia (CLL) often have marked but transient increases of circulating CLL lymphocytes following treatment with PCI-32765, as has been seen with other inhibitors of the B cell receptor (BCR) pathway. In the course of the Phase I study of PCI-32765, we have noted similar effects among treated patients with other types of non-Hodgkin lymphoma (NHL) including mantle cell lymphoma (MCL). We here characterize the patterns and phenotypes of cells mobilized among patients with MCL, and further investigate the mechanism of this effect. Nine patients with MCL treated in the previously reported Phase I study (Advani et al, ASCO, 2010) had baseline absolute lymphocyte counts (ALC) of 1.04 ± 0.42 (x 10^9/L, Mean ± SD) and had maximal increases during the first 28 day cycle of 12 to 794% (188% increase ± 250, Mean, SD). The ALCs of four patients who were treated on a dosing schedule that included a 1 week drug holiday within each cycle were noted to show intra-cyclic increases of ALC from day 1 to day 15 of each cycle, and decreases following each week off of treatment, for up to 9 cycles (Fig. 1). Patients receiving continuous dosing exhibited gradually decreasing ALCs following the first cycle. The cyclically increasing B lymphocytes were confirmed to be $CD5^+$ (and often also $CD45^{lo}$), and thus likely to represent circulating, mobilized lymphoma cells. Patient, D005, who attained a complete response, had an easily identifiable $CD19^+CD45^{lo}$ subpopulation of 0.47 x 10^9 cells/L at baseline. This subpopulation increased to 15.2 x 10^9/L at day 8 of the first cycle, but then decreased markedly as the patient responded clinically. One patient who failed to respond had, by contrast, few if any detectable mobilized cells. Peripheral blood $CD19^+CD5^+$ cells from MCL patients treated with PCI-32765 after 8 days were found to have reduced levels of CXC chemokine receptor 4 (CXCR4) levels, whereas pretreatment malignant cells were $CXCR4^{hi}$. This likely reflects the differences in MCL surface membrane phenotype in solid tissues compared to peripheral blood. Mechanistically, we found that PCI-32765 inhibited BCR- and CXCL12-mediated adhesion and chemotaxis of MCL cell lines in vitro (EC_{50} = 10-100 nM), and dose-dependently inhibited BCR, stromal cell and CXCL12 stimulations of pBtk, pPLCg and pErk in MCL cells. Importantly, PCI-32765 dose-dependently inhibited the pseudoemperipoleisis of MCL in the presence of stromal cells.

Conclusion: Lymphocyte mobilization into the peripheral blood is notable from MCL in response to treatment with PCI-32765. The majority of these cells are marked with a phenotype ($CD19^+CD5^+$ $CXCR4^{lo}$) which is consistent with malignant cells from secondary lymphoid organs. This effect is likely to be related to PCI-32765 inhibition of BTK activation which results in inhibition of MCL cell chemotaxis, adherence and pseudo-emperipoleisis. We propose that Btk is essential for the homing of MCL cells into secondary lymphoid organs, and that its inhibition results in peripheral blood compartment shift.

Title: Activity of Bruton's tyrosine kinase (Btk) inhibitor PCI-32765 in mantle cell lymphoma (MCL) identifies Btk as a novel therapeutic target (ASH 2011 Annual Meeting Abstract #3688)

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B cell receptor (BCR) signaling is critically involved in the progression of several B cell malignancies, but its role in mantle cell lymphoma (MCL) remains incompletely defined. Bruton's tyrosine kinase (Btk) is a central regulator of BCR signaling and can be selectively and irreversibly inhibited by PCI-32765, which is emerging as a new, molecularly targeted therapy for patients with B cell malignancies. In this study, we explored the role of Btk and the activity of PCI-32765 on BCR signaling in several MCL lines, including Granta-519, Jeko-1, JVM-2, JVM-13, Maver-1, Mino, NCEB-1, Rec-1 and Z-138. Btk and surface IgM protein expression was detected in all MCL lines at variable levels. In a 3-day proliferation assay, JVM-2 & MINO emerged as the most sensitive lines to PCI-32765 (GI50: 1.75-4.4μM). Rec-1 was resistant to PCI-32765 alone (11.3μM) but became much more sensitive (1.45μM) upon BCR stimulation using anti-IgM (10μg/mL). Other lines such as Maver-1, Granta-519 & Jeko-1 all required >10μM of PCI-32765 for inhibition and BCR stimulation did not make much difference.

When signaling pathways downstream of BCR activation were studied, intracellular calcium flux following stimulation with IgM was observed in all lines (except JVM-2) and was inhibited at <100nM PCI-32765 in most of them, but no correlation between this and growth inhibition was observed. Constitutive BTK autophosphorylation was observed in all lines and was completely abolished by PCI-32765. BCR stimulation increased p-BTK which was also blocked by PCI-32765 in all lines. Mino and JVM-2 showed constitutive p-ERK activity, which was slightly increased upon BCR stimulation and could be blocked with PCI-32765, whereas the more resistant lines such as Maver-1 and Rec-1 had low endogenous levels of p-ERK, but which was increased by BCR stimulation and only partially or not reversed by PCI-32765 at 5μM. Little change was observed in levels of p-PLCg1 or p-NF-κB p65.

Additionally, in all cell lines stimulation with anti-IgM led to an increased secretion of the chemokines CCL3 and CCL4, which are surrogate biomarkers for BCR-derived activation of neoplastic B cells (Burger JA et al., *Blood* 113:3050-8, 2009) with greatest increase in JVM-13 and Rec-1 cells. Pre-treatment of these two MCL lines with PCI-32765 significantly inhibited CCL3 and CCL4 secretion in a dose dependent fashion with total abrogation of chemokine secretion at concentrations of 10 μM PCI-32765 (see Figure).

Early clinical data indicate that PCI-32765 induces a rapid reduction in lymphadenopathy accompanied by a transient lymphocytosis (in CLL, but also in MCL patients), presumably due to mobilization of the malignant B cells from the tissue compartments into the peripheral blood. Therefore, we analyzed the effect of PCI-32765 (conc. 0.5 and 1 μM) on MCL responses to a lymph node homing chemokine, CXCL13. We found that CXCL13-induced actin polymerization in Rec-1 cells was significantly reduced by PCI-32765, even at lower concentration.

We conclude that MCL cells express functional Btk, which is involved in BCR signaling in MCL cells. Blockade of Btk function using PCI-32765 inhibits MCL cell proliferation, BCR signaling, chemokine secretion, and interferes with MCL cell actin polymerization. These findings highlight the importance of BCR signaling and Btk in MCL, help explain the activity of the Btk inhibitor PCI-32765 in MCL patients, and provide biomarkers that may be of value in the clinic.

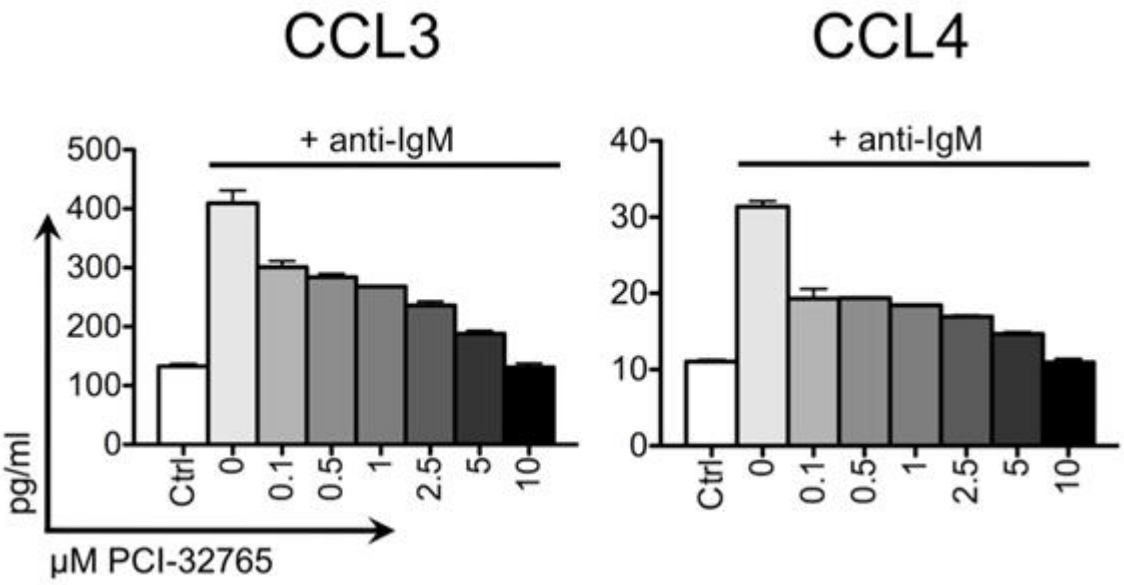

Figure: CCL3 and CCL4 concentrations in cell culture supernatants of the MCL cell line JVM-13 after 24 hour stimulation with anti-IgM and abrogation of this stimulation after pre-treatment with PCI-32765.