

Session 14: signalling pathways in lymphoma

151 INTRODUCTORY LECTURE

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Diffuse large B-cell lymphoma (DLBCL), the most common lymphoid malignancy in adults, is currently treated with empiric combination chemotherapy and rituxan. To obtain additional insights into pathogenetic disease mechanisms and potential approaches to more targeted therapy, our group has used comprehensive genomic approaches including transcriptional profiling and, more recently, copy number analysis. These studies identified a group of DLBCLs that are more reliant upon B-cell receptor survival signals and more sensitive to targeted inhibition of proximal components of the BCR-signaling cascade.

The genomic analyses of primary tumor samples and the preclinical evaluation of informative cell lines and viable primary tumor specimens led to a recently completed clinical trial in which a subset of heavily pretreated relapsed/refractory DLBCLs and additional B-cell lymphomas responded to targeted BCR inhibition.

In additional studies, integrative analyses of high-resolution copy number data and transcriptional profiles identified highly significant perturbations of targetable signaling pathways in DLBCL. These newly identified genetically perturbed signaling pathways and additional insights into the specificity and mechanisms of targeted inhibition of BCR signaling will be discussed in further detail.

152 MULTICENTER PHASE II TRIAL OF MLN8237, AN INVESTIGATIONAL INHIBITOR OF AURORA A KINASE (AAK), IN PATIENTS (PTS) WITH AGGRESSIVE B-CELL AND T-CELL NON-HODGKIN LYMPHOMA (NHL)

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Background: AAK is a serine-threonine kinase that regulates mitosis and multiple signaling cascades. AAK inhibition leads to mitotic errors, chromosomal aneuploidy and subsequent cell death by apoptosis or senescence. AAK is overexpressed in several tumors and aggressive lymphomas. Investigational drug MLN8237 is an ATP-competitive, oral inhibitor of AAK. We conducted a phase II trial of MLN8237 in patients with aggressive B- and T- cell NHL.

Methods: Eligible pts had normal organ function, ANC $\geq 1250/\text{mm}^3$, platelets $\geq 75,000/\text{mm}^3$. Pts received MLN8237 50mg BID for 7 d on 21 d cycles.

Results: 48 pts were enrolled, and 40 are evaluable for response (Table). Histologies included DLBCL (n=21, 44%), mantle cell (MCL; n=13, 27%), peripheral T cell (n=8, 17%), transformed follicular (n=5, 10%) and Burkitt's (n=1, 2%). Median age was 67.5 y (range 32–85). Pts received median 3 prior regimens (range 1–11); 11 pts received prior ASCT. Most common adverse events (AEs) were neutropenia (71%), fatigue (60%), leukopenia (58%), diarrhea (56%), anemia (56%), and alopecia (48%). Serious AEs in $\geq 5\%$ of pts were febrile neutropenia (15%); neutropenia (13%), stomatitis (10%), pneumonia (6%) and deep vein thrombosis (6%). Four deaths on study were related to progressive NHL (2), treatment-related sepsis (1), and unrelated cause (1). 8 pts discontinued and 24 reduced dose to 40mg BID due to AEs. The overall response rate (ORR) was 29% (95% CI 0.166–0.465): 5 CR (3 T-cell; 1 MCL and 1 Burkitt) and 9 PR including 3/11 post-ASCT. 5 pts remain on treatment including 1 near PR (PTCL).

Conclusion: These findings suggest MLN8237 has antitumor activity in pts with NHL including heavily pretreated and relapsed post-transplant. Data supporting this conclusion was observed across all histologies, particularly aggressive T-cell. Toxicities were generally manageable and reversible; 4 pts continued for ≥ 1 y. Future trials are planned, including in aggressive B- and T-cell NHL.

Histology	DLBCL	T cell	MCL	Transformed Follicular	Burkitt
N	15	6	13	5	1
CR/PR	0/3	3/0	1/2	0/2	1/0
SD	6	2	6	2	0
PD	6	1	4	1	0

153 THE BTK INHIBITOR PCI-32765 IS HIGHLY ACTIVE AND WELL TOLERATED IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES: FINAL RESULTS FROM A PHASE I STUDY

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Introduction: PCI-32765 is a potent, selective, irreversible small molecule inhibitor of Bruton's tyrosine kinase (Btk), a downstream mediator of B-cell receptor signaling.

Methods: PCI-32765 was administered orally with dose escalation according to protocol-defined dose-limiting toxicities to define a maximum tolerated dose (MTD) or until 3 dose levels above attainment of full Btk occupancy. A 28-day on / 7-day off schedule was evaluated in 5 cohorts (1.25–12.5 mg/kg po qd) and a once daily oral dose (without a drug holiday) in 2 cohorts (8.3 mg/kg and 560-mg fixed dose). Response was evaluated every 2 months according to standard criteria for NHL and CLL.

Results: 56 pts with relapsed/refractory disease [median 3 prior regimens (range 1–10)] were enrolled. Possible related grade (gr) ≥ 3 adverse events occurred in 16% of pts with minimal (<7%) gr ≥ 3 neutropenia or thrombocytopenia. No renal/hepatic toxicity, treatment-related deaths, or cumulative toxicity with prolonged dosing was observed. No dose-limiting toxicity was identified at any dose level and the MTD not reached. Pt cohorts treated at ≥ 2.5 mg/kg exhibited complete Btk occupancy as assessed by a competitive binding assay in PBMCs. Mean half life ranged from 6–11 hours (h). Basophil degranulation, a Btk-dependent cellular process, was completely inhibited ≤ 24 h post dosing. 30/50 (60%) of evaluable pts achieved an objective response [complete response (CR) n=7, partial response (PR) n=23] seen at all dose levels and across all histologies (Table 1). At a median follow-up of 6 months (range <1–19), the median response duration for CLL, MCL, FL, and WM has not been reached. 25 pts remain on study without disease progression.

Histology	n	Objective Response n (%)
Chronic lymphocytic leukemia (CLL) / Small lymphocytic lymphoma (SLL)	14	11 (79)
Follicular lymphoma (FL)	13	6 (46)
Mantle cell lymphoma (MCL)	9	7 (78)
Diffuse large B cell lymphoma (DLBCL)	7	2 (29)
Waldenström macroglobulinemia (WM)	4	3 (75)
MALT or marginal zone lymphoma	3	1 (33)

Conclusion: PCI-32765 is a novel, well tolerated oral irreversible Btk inhibitor with significant durable responses across various B cell histologies. Phase II trials of single-agent PCI-32765 in CLL, MCL, and DLBCL are ongoing and studies in combination with standard therapies planned.

154 PIM INHIBITION AS A RATIONAL THERAPEUTIC APPROACH IN DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction/Background: The PIM kinases are a family of serine/threonine kinases involved in the phosphorylation and regulation of several proteins that

are essential for cell cycle progression, metabolism and apoptosis (BAD, p21, AKT, cMyc.). Overexpression, translocation or amplification of PIM family genes have been described in many human cancers, including B-cell non-Hodgkin's lymphoma, multiple myeloma and prostate cancer. In addition, 50% of patients diagnosed with diffuse large B-cell lymphoma (DLBCL) present somatic mutations in *PIM1* as a result of aberrant somatic hypermutation, while *PIM1* and *PIM2* increased expression distinguish ABC-DLBCL. Despite its role in the regulation of pathways considered as relevant in cancer, very few chemical inhibitors have been described. The aim of this study is to investigate whether PIM inhibition is a rational therapeutic approach for treating DLBCL, developing tools for patient stratification and in pharmacodynamic studies.

Material and Methods: Gene expression profiling was conducted in a series of 22 DLBCL patients. *PIM2* immunohistochemical expression was assessed in an additional subset of 176 DLBCL samples. The effect of PIM inhibition was checked on cell lines by using specific genetic (siRNA) and pharmacological inhibitors (ETP-39010). Newly produced antibodies and protocols were standardized.

Results: Gene expression data revealed high *PIM2* expression in a subset of patients with ABC-DLBCL subtype. *PIM2* protein expression was immunohistochemically assessed in an independent series of 176 homogeneously treated DLBCL patients, revealing a strong positivity for *PIM2* in a 23% (41/176) of DLBCL cases. Correlation of *PIM2* protein expression with clinical outcome, revealed a shorter Overall Survival ($p=0.001$) in patients positive for *PIM2* expression. When DLBCL cases were divided into GC and ABC phenotypes, the difference in survival probability was shown to be restricted to those of the ABC-DLBCL subtype. Lymphoma cell lines (10) derived from both ABC-DLBCL and GC-DLBCL subtypes were analyzed by qRT-PCR and Western-blot, showing high levels of *PIM2* in cell lines characteristic of the ABC-subtype. Pharmacological and genetic inhibition of *PIM2* induced cell cycle arrest, revealing p4E-BP1 (S65) and p4E-BP1 (T37) as molecular biomarkers characteristic of *PIM2* activity.

Conclusion: We show that *PIM2* inhibition is a rational approach in DLBCL treatment, identifying appropriate biomarkers for pharmacodynamic studies, and providing a new marker for patient stratification.

155 TARGETING CAP-DEPENDENT TRANSLATION TO BYPASS PRO-SURVIVAL SIGNALING BY AKT AND THE PIM FAMILY KINASES IN NON-HODGKIN LYMPHOMA

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Introduction/Background: AKT and the Pim family kinases promote cell survival in both normal and malignant lymphocytes. The pathways have many substrates in common and are frequently activated in multiple sub-types of non-Hodgkin lymphoma. Importantly, the pathways converge on activation of eIF4E-mediated cap-dependent translation. AKT does so in a rapamycin-sensitive manner downstream of mTOR, while Pim does so in a rapamycin-resistant manner. We explore the hypothesis that targeting cap-dependent translation directly may bypass both pathways simultaneously.

Materials and Methods: We have compared AKT and Pim in multiple lymphoma-specific *in vitro* and *in vivo* laboratory systems. We have made extensive use of small-molecule inhibitors, including rapamycin, the Pim kinase inhibitor SG-1773, and silvestrol, which disrupts cap-dependent translation.

Results: We find that activation of AKT and/or Pim activity may be an inherent property of NHL tumors or may result from cytokine stimulation and may therefore be an important mediator of therapy resistance provided by the tumor microenvironment. Studies *in vivo* demonstrate Pim's ability to accelerate oncogenesis in a manner similar to AKT in models of both aggressive lymphoma (E μ -Myc) and indolent lymphoma (VavP-Bcl2). Treatment studies show that Pim promotes resistance to anthracycline chemotherapy like AKT. However, Pim tumors completely resist rapamycin in stark contrast to AKT tumors. Further studies show that Pim's ability to mediate rapamycin resistance is dependent on its ability to maintain inhibitory phosphorylation of the translation repressor 4EBP1. We have expanded on this finding using silvestrol, which shows high potency against Pim-expressing murine tumor cells *in vitro* (IC₅₀ < 1 nM) and *in vivo*. Silvestrol also shows potency against a panel of Pim-expressing human lymphoma cell lines (IC₅₀ 1-10 nM).

Conclusions: In conclusion, multiple mechanisms may lead to deregulated activity of pro-survival kinases in NHL, including cytokine stimulation by the tumor microenvironment. The benefit of targeting mTOR activity downstream of AKT is lost in the context of Pim expression, but directly targeting cap-dependent translation potentially bypasses both pathways simultaneously. Clinical trials of drugs with activities like silvestrol's therefore are warranted to potentially improve treatment options for NHL patients.

156 A NOVEL SMALL MOLECULE MEK INHIBITOR INDUCES CELL DEATH THROUGH A BIM-DEPENDENT MECHANISM IN NON-HODGKIN LYMPHOMA (NHL) CELL LINES, PRIMARY CELLS, AND A HUMAN NHL XENOGRAFT MODEL

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Introduction: Potent small-molecule inhibitors of the RAS/RAF/MEK/ERK MAPK pathway have been developed, however, the majority of data have emerged mainly from solid tumor studies. Further, targeting MEK/ERK is unexplored in NHL. AZD6244 is a novel 2nd generation small molecule MEK antagonist being developed for the treatment of cancer.

Methods: Diffuse large B-cell lymphoma (DLBCL) cell lines (SUDHL4, SUDHL6, SUDHL10, OCI-LY3, and OCI-LY19) and primary cells (from 3 patients with relapsed, transformed DLBCL) were incubated with nanomolar concentrations of AZD6244 (50-400nM). Apoptosis was determined by AnnexinV-FITC/propidium iodide (AnnV⁺/PI⁺) staining. Multiple kinase substrates, including phosphorylated ERK (pERK) and pMCT-1, were assessed through Western blot, while OCI-LY3 cells were transfected with BIM siRNA using the Amaxa Nucleofector kit V. *In vivo* studies were performed with mice bearing SQ SUDHL6 tumors/cells that were subcutaneously injected into the bilateral dorsal flanks of 7-week-old female SCID mice. When the tumor reached 60-163mm³, AZD6244 was administered intra-peritoneally every other day at 1.0 mg/kg for 3 weeks.

Results: Time-dependent cytotoxicity was documented in each of the 5 DLBCL cell lines. IC₅₀ at 48 hours (hrs) in SUDHL4 and OCI-LY3 was 130nM, 240nM in SUDHL6 and SUDHL10, and 300nM in OCI-LY19. Dose-dependent apoptosis was also seen in all cell lines; compared with control at 48 hrs, >50% AnnV⁺/PI⁺ was documented in SUDHL-4 at 200nM and with 300nM in the 4 other cell lines ($p<0.01$). Further, 100nM of AZD6244 effectively down-regulated pERK in each cell line, while 200-300nM was required for significant down-regulation of pMCT-1. In primary DLBCL cells, dose-dependent apoptosis was also noted (e.g., 100nM AZD-6244: >75% AnnV⁺/PI⁺ ($p<0.001$)). AZD6244 induced apoptosis was associated with: activation of cleaved PARP and caspases-8, -9, and -3; activation of BH-3 protein BIM; activation of p27; and inhibition of FOXO3a and MCL1. Moreover, blockade of BIM utilizing siRNA significantly abrogated AZD6244 apoptosis ($p<0.01$). Finally, with *in vivo* studies, mice treated with AZD-6244 from 28 weeks on had significantly (and increasingly) less average tumor volume compared with control ($p<0.05$).

Conclusion: Collectively, these data show that the novel anti-MEK small molecule AZD6244 induced significant cell death at nanomolar and clinically achievable concentrations in DLBCL cell lines, primary cells, and in an *in vivo* xenograft model that occurred through a BIM-dependent mechanism.

157 PHASE 1 STUDY OF A NOVEL ORAL JAK2 INHIBITOR, SB1518, IN PATIENTS WITH RELAPSED LYMPHOMA: EVIDENCE OF CLINICAL AND BIOLOGIC ACTIVITY IN MULTIPLE LYMPHOMA SUBTYPES

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Introduction: The JAK2-STAT pathway plays an important role in the pathogenesis of hematologic malignancies. *In vitro* inhibition of JAK2 results in antiproliferative activity in a variety of lymphoma cell lines. We conducted a Phase 1 study of SB1518, a potent JAK2 inhibitor, in patients with relapsed lymphoma.

Methods: The main objectives were to establish the MTD of SB1518 and assess its safety, tolerability, PK, and PD. Eligible patients had relapsed or refractory Hodgkin (HL) or Non-Hodgkin lymphoma (NHL), any number of prior treatment regimens, and adequate organ function and performance status. Cohorts of 3-6 patients received escalating dose levels of SB1518 orally once a day for 28-day cycles. Response was first evaluated after 8 weeks.

Results: Thirty-four patients were enrolled (14 HL, 10 follicular [FL], 5 mantle cell [MCL], 4 diffuse large B-cell, 1 small lymphocytic [SLL]); 30 were treated at 5 dose levels (100-600 mg/d). Dose-limiting toxicities were Gr 4 neutropenia (1 episode, 300 mg/d) and Gr 4 thrombocytopenia (1 episode, 400 mg/d). The MTD was not reached. Enrollment at 600 mg/d was expanded to confirm safety at this dose level. Treatment was well tolerated, with mostly Gr 1/2 toxicities. GI toxicity (diarrhea [37%], constipation [30%], nausea/vomiting [23%], decreased appetite [13%]) were the most common treatment-related events. Gr 3/4 non-hematologic toxicity was uncommon. Overall, cytopenias were infrequent and modest. There were 3 PRs (2 MCL, 1 FL at 300, 400, 600 mg/d) and 15 SDs (7 FL, 6 HL, 1 MCL, 1 SLL), with the majority of responses sustained for > 2 months; 7/13 patients with SD had tumor mass reductions of 4-46%. Pharmacologically active concentrations were achieved at the lowest dose level. Dose-related increases in AUC were seen on Day 1 and Day 15 up to the 400 mg/d level; the mean terminal half-life was 1-3 days, and mean T_{max} ranged from 5-9 hours. SB1518 inhibited JAK2 signaling as early as 4

hours after the first dose at all dose levels. Increases in Flt3-L (reflecting FLT-3 inhibition) and G-CSF (reflecting JAK2 inhibition) were seen in a majority of patients.

Conclusions: SB1518 has encouraging activity in patients with relapsed lymphomas; clinical benefit was observed in several lymphoma subtypes at doses \geq 300 mg/d and was well tolerated at doses up to 600 mg/d. The recommended dose for Phase 2 study is 400 mg/d. A Phase 2 trial of SB1518 in selected lymphomas is ongoing.

158 A PHASE 1 STUDY OF CAL-101, AN ISOFORM-SELECTIVE INHIBITOR OF PHOSPHATIDYLINOSITOL 3-KINASE P110 δ , IN COMBINATION WITH ANTI-CD20 MONOCLONAL ANTIBODY THERAPY AND/OR BENDAMUSTINE IN PATIENTS WITH PREVIOUSLY TREATED B-CELL MALIGNANCIES

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Background: The class I phosphatidylinositol 3-kinases (PI3Ks) regulate cellular functions relevant to oncogenesis. CAL-101 is an isoform-selective

inhibitor of PI3K p110 δ isoform (PI3K δ) that has demonstrated substantial single-agent clinical activity in patients with B-cell malignancies.

Methods: This Phase 1 study has evaluated CAL-101 in combination with rituximab or bendamustine in patients (pts) with previously treated indolent non-Hodgkin lymphoma (iNHL) and chronic lymphocytic leukemia (CLL). All pts received CAL-101 100 or 150 mg orally twice per day (BID) in 28-day cycles for up to 12 cycles. CAL-101 was administered starting on Day 1 of Cycle 1 with rituximab 375 mg/m² given weekly for 8 weeks (RC regimen), or bendamustine 90 mg/m² given on Days 1 and 2 of each cycle for 6 cycles (BC regimen). Tumor response was evaluated according to standard criteria.

Results: At data cutoff, pt accrual was 28 for iNHL and 21 for CLL. Pt characteristics included: median age [range] of 64 [41-87] years; 65% males; 43% with refractory disease; and a median [range] of prior therapies of 3 [1-9]. 24 pts received CAL-101 100 mg BID (12 RC/12 BC) and 25 pts received 150 mg BID (13 RC/12 BC). Grade \geq 3 adverse event rates have been as expected: including neutropenia and thrombocytopenia each in 10/24 pts receiving BC, and increased ALT/AST in 6/28 pts with iNHL and 1/21 in pts with CLL. Among 35 evaluable pts, there were preliminary overall response rates of 19/22 (9/10 RC, 10/12 BC) in pts with iNHL, and 9/13 (4/6 RC, 5/7 BC) in pts with CLL. Compared to baseline, on-treatment peripheral lymphocyte counts were stable or decreased in 17/21 pts with CLL. Accrual is ongoing and updated data will be presented.

Conclusions: Early results from this Phase 1 study of CAL-101, an oral PI3K δ isoform-selective inhibitor, administered in combination with rituximab or bendamustine, show acceptable safety and promising clinical activity in pts with previously treated iNHL and CLL.