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Results of PX-171−003-A0, part 1 of an open-label, single-arm, phase 2 study of carfilzomib in patients with relapsed and refractory multiple myeloma

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Background: Carfilzomib (CFZ) is a novel proteasome inhibitor of the epoxyketone class that exhibits a high level of proteasome selectivity and demonstrates antitumor activity in bortezomib (BTZ)-resistant multiple myeloma (MM) patients (pts) in phase 1 studies.
Methods: PX-171−003-A0 was an open-label, multicenter study that enrolled MM pts who relapsed from >2 prior therapies, failed BTZ and at least 1 immunomodulatory agent [thalidomide (THAL) or lenalidomide (LEN)], and were refractory to last treatment [progressing on or within 60 d of last therapy or <25% response to last therapy]. Pts received CFZ 20 mg/m^2 IV d 1, 2, 8, 9, 15 and 16 every 28 d for up to 12 cycles (C). Clinical benefit response (CBR) was defined as MR or better.
Results: 46 pts were enrolled, including 78% with progression on or within 60 d of last therapy and 22% with no response to last therapy. 39 pts completed at least 1 C of CFZ, had measurable M-protein, and were evaluable for response. Median prior therapies was 5 (range 2−15). 100% of pts received prior BTZ, 91% prior THAL, 89% prior LEN, and 83% prior stem cell transplant (SCT) and all had failed combinations including anthracyclines (80%) and/or alkylating agents (94%). Pts received a median of 3 C (range 1−12); 13 pts completed ⩾6 C. CBR was 26% (10/39 eval pts), including 5 pts achieving PR and 5 pts achieving MR. 5 BTZ-refractory pts achieved MR or PR. Median TTP was 6.2 mo, the median DOR for the MR + PR was 7.4 mo. 8/10 pts achieved response during C1. 16 additional pts achieved SD for at least 6 wks. The most common adverse events were fatigue, anemia, thrombocytopenia, nausea, upper respiratory infection, increased creatinine and diarrhea. Peripheral neuropathy occurred in <10% of pts with 1 Gr 3 in a pt with pre-existing Gr 2. The FACT/GOG-NTX QOL was improved over baseline.
Conclusions: Single-agent CFZ achieved a TTP of > 6 mo in relapsed and refractory MM pts who failed available therapies. 26% of patients had at least an MR and median duration of >7 mo with this steroid- and anthracycline-sparing regimen. CFZ toxicities were manageable and importantly, exacerbation of pre-existing PN was rare. The study has been expanded to enroll an additional 250 pts in this unmet medical need population at an stepped-up dose schedule of 20/27 mg/m^2.

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Array based CpG island methylation-profiling in acute myelogenous leukemia at diagnosis and relapse

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Background: In acute myelogenous leukemia (AML), pathological hematopoietic progenitor or stem cells show uncontrolled proliferation and arrest in maturation. Cytogenetic analyses have identified specific recurrent chromosomal aberrations. Additionally, methylation of cytosines in the promoter region of many genes is involved in regulating gene expression. In this study we used the Illumina GoldenGate© methylation assay to assess the methylation status of a large number of selected genes in 32 AML patients at diagnosis and relapse, compared to normal controls.
Material and Methods: We obtained 31 bone marrow (BM) and one peripheral blood specimen during routine clinical assessment of 32 patients with newly diagnosed AML treated at the University Hospital Aachen (Germany) from 1997 to 2008 and used 11 non-malignant BM specimens and four peripheral stem cell harvests as controls. From nine of the 32 AML patients, BM at time of relapse was also available. The methylation status of 1505 CpG-sites from 807 genes was simultaneously determined using the GoldenGate© Methylation Cancer Panel I assay (Illumina, San Diego, CA, USA), which provides a continuous measure of methylation density ("β-value" between 0.0 and 1.0) of each site. For comparison, methylation-specific PCR (MSP) was performed for two genes (SFRP1 and CDKN2B).
Results: Cluster analysis of array results revealed a similar methylation profile among the normal controls compared to AML samples at diagnosis. Within the AML samples, an association between methylation patterns and recurrent cytogenetic aberrations such as del(5), del (7), inv (16) and t (8;21) could also be found. Overall, methylation in AML samples was higher than in the controls (mean β-value 0.3449 vs. 0.3084). We identified 216 CpG-sites that were mostly unmethylated in controls but yielded significantly higher methylation in the AML samples. For 12 sites, a significant correlation between methylation status and survival could be detected. Comparing the nine corresponding samples at diagnosis and relapse, only small changes in the methylation profile and a slight increase in overall methylation (0.3491 vs. 0.3361) were detected at time of relapse. Additionally, we found a strong correlation between the results of array analysis and MSP.
Conclusions: Hypermethylation is a frequent event in AML and is accentuated at relapse. Array-based methylation analysis of a large number of genes determined distinct methylation profiles for normal controls and AML samples with specific chromosomal aberrations. The methylation status of several specific genes had a significant impact on survival.

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Lipoxin A4 accelerates the resolution of acute promyelocytic leukemic cells in retinoic acid syndrome

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Background: Retinoic acid syndrome (RA syndrome) may develop in patients with acute promyelocytic leukemia (APL) during treatment with all trans retinoic acid (ATRA), which is characterized by which is characterized by massive infiltration of ATRA-treated APL cells into alveolar spaces. Resolution phase of RA syndrome has not been studied. Lipoxin A4 is an anti-inflammatory mediator during acute inflammation and its role in RA syndrome is still unknown.
Materials and Methods: We determined the crosstalk between the ATRA-treated APL (NB4) cells and alveolar macrophages (NR8383 cells) by transmigration assay, phagocytosis assay and ELISA.
Results: Condition medium (CM) of co-culture of alive NR8383 cells and dead ATRA-treated NB4 cells can inhibit the transmigration of alive ATRA-treated NB4 cells in a dead NB4 cells dose-dependent manner. The level of lipoxin A4 in the CM increased when a fixed number of alive NR8383 cells were co-cultured with increased cell number of dead ATRA-treated NB4 cells. Lipoxin A4 itself can inhibit the transmigration of ATRA-treated NB4 cells in a dose dependent manner. Receptor of lipoxin A4 (FPRL-1) was expressed in both ATRA-treated NB4 cells and NR8383 cell by immunohistochemical stain and flowcytometry. BOC-2 (inhibitor of FPRL-1) can further increase the transmigration of ATRA-treated NB4 cells, indicating the active role of lipoxin A4 in the ATRA-treated NB4 cells. We also demonstrated that lipoxin A4 can enhance the phagocytosis of dead ATRA-treated NB4 cells by NR8383 cells.
Conclusion: Lipoxin A4 can inhibit the transmigration of ATRA-treated APL cells and increase the phagocytic activity of alveolar macrophages. This indicates that lipoxin A4 contributes an important role in the resolution phase of RA syndrome.

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Polymorphisms of genes MDR1 and MTHRF in children with acute leukemia

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Background: The objective of this study is to evaluate the polymorphism frequency of MDR1 (multidrug resistance) and MTHRF (5,10-methylenetetrahydrofolate reductase) genes which can modulate the risk of acute leukemia.
Material and Methods: The study included 30 patients with acute myeloid leukemia (AML), 40 patients with acute lymphoblastic leukemia (ALL) and control group composed of 31 individuals without leukemia. The age of children ranged from 0 to 22 years (median 7 years). Gender distribution

was follows: in ALL group there were 27 (67.5%) male and 13 (32.5%) female patients; in AML – an equal proportion of males and females – 15 (50.0%); in control group 18 (69.2%) males and 8 (30.8%) females.
For MDR1 C3435T and G2677T polymorphisms analysis allele-specific real-time PCR assay was used. For detect MTHFR C677T gene polymorphism we used restriction fragment length polymorphism assay.
Results: The analysis of genotype frequencies showed that for MDR1 G2677T and MTHFR genotypes there is no statistically significant difference between groups of patients with AL and the control. But the analysis of the distribution of MDR1 C3435T gene polymorphic variations did reveal statistically significant differences between the studied groups. The TT genotype was prevalent in children with ALL 20.0% ($p = 0.008$) and AML 16.7% ($p = 0.02$) and was absent in control group. The control group was predominantly MDR1 C3435T heterozygous – 87.7% – which makes a statistically significant difference in comparison with the ALL 57.5% ($p = 0.009$) and AML 60.0% ($p = 0.02$) groups.
The analysis of the gene polymorphism distribution between male and female patients has shown no significant difference in frequency for all groups except the control one in which the majority of females had the MDR1 G2677G homozygous genotype 76.9% ($p = 0.004$), while half of males had the MDR1 T2677T homozygous mutant genotype 50.0% ($p = 0.02$). The analysis of research literature showed that the majority of studies point to the lack of correlation between gender and genotypes of investigated genes.
Conclusions: There were statistically significant differences in the distribution of MDR1 C3435T genotypes between the AL and control groups and of G2677T variations – between females and males. In the first case, the mutant TT genotype was detected only in children with leukemia, and heterozygosity was characteristic for the control group. Differences in the distribution of G2677T polymorphism between males and females may be random in nature, due to the small sample size and require further study.

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Imatinib plus vincristin & prednisolone induces complete remission and prolonged survival in elderly philadelphia chromosome positive acute lymphoblastic leukemia patients

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Introduction: Acute lymphoblastic Leukemia (ALL) in elderly patients (50yrs or older) carries a poor prognosis. In survival studies using in variety of therapeutic regimens. This may be because of relatively high frequency of the Philadelphia chromosome (Ph). With the advent of dose intensive chemotherapy regimen such as hyper CVAD (Fractionated Cyclophosphamide, Vincristin, Doxorubicin, Dexamethasone) overall survival has not improved. The aim of our study was to see the effectiveness of Imatinib plus Vincristin & Prednisolone in Philadelphia Chromosome positive in elderly acute lymphoblastic leukemia patients.
Material and Methods: During period from January 2006 December 2008 we selected 30 consecutive elderly (more than 50yrs) Bcr-Abl ALL patients in the haemato-oncology department Netaji Subhash Chandra Bose Cancer Research Institute. There were 12 males & 18 females. The median age of the patient was 64 years (range 51 to 77yrs). All patients were started with Imatinib mesylate (Natco pharma) 400 mg daily. Prednisolone was given 40 mg /m^2 over 6weeks & followed by 2weeks tapering dose. Vincristin was given 2 mg/m^2 weekly for 6weeks. All patients were evaluated by bone marrow and Bcr-Abl estimation by flowcytometer every 3 months for 1 year then 6 monthly.
Result: 24 patients (80%) obtained complete haematological & partial molecular response at 3month. Nine patients (30%) achieved complete molecular response at 9month. With median follow-up of 12 months (range 4−20months) the disease free survival and overall survival were 60% & 70% respectively. Most of the induction treatment was done as OPD basis, no hospitalization required. The therapy was tolerated well.
Conclusion: We concluded that Imatinib plus Vincristin & Prednisolone is a feasible, highly active protocol for elderly Bcr-Abl positive acute lymphoblastic leukemia patients. It is well tolerated & associated with good quality of life.

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Arsenic trioxide induces apoptosis in NB-4, an acute promyelocytic leukemia cell line, through up-regulation of p73 via suppression of nuclear factor kappa B-mediated inhibition of p73 transcription

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Background: Acute promyelocytic leukemia (APL) is characterized by t(15;17). Although Arsenic trioxide (ATO) is the treatment of choice in APL, the molecular mechanisms underlying its anti-proliferative effects are not fully understood. p73 is a new member of p53 family capable to transactivate p53-responsive promoters to induce cell cycle arrest and apoptosis. NF-κB contributes to ubiquitin-dependent proteasomal degradation of p73, inhibits its transcription and proapoptotic functions. Given this, therapeutic disabling of NF-κB might promote apoptosis in tumor cells through triggering p73-mediated apoptosis. This study was aimed to evaluate the effects of ATO on transcriptional activity of p73 through preempting NF-κB.
Material and Methods: MTT assay, BrdU-proliferation assay, Caspase 3 assay, Cell-based NF-κB phosphorylation assay by ELISA and real time PCR array were employed to assess the effect of ATO on proliferation of NB-4 cells, activation of pro-caspase 3, phosphorylation of NF-κB cascade and expression of genes involved in survival as well as stabilization and degradation of p73.
Results: ATO suppressed proliferation of NB-4 cells, activated pro-caspase 3, hindered phosphorylation of p65 subunit of NF-κB and caused an increase in transcriptional activity of p73 and its targets p21 and TP53INP1. Considerable reduction in transcriptional levels of IKK2 and Nemo, subunits of IKK complex, and ATM (which activates NF-κB) and TIP60 (which represses p73 message) was observed. Moreover, ATO repressed mRNA levels of XIAP, BCL-XL, IAP1, Bcl-2 and survivin. No inhibitory effect of ATO on mRNA expression of IAP2, cyclin D1 and BFL2 was obtained. ATO exerted no inductionary effect on promoter activity of CYLD and ING4, repressors of NF-κB, neither Kpm nor CD145 and YAP1 which stabilize p73. No inhibitory effect of ATO on Itch (which governs p73 proteasomal degradation as well as reduces its mRNA message)was obtained.
Conclusions: The results of the present study imply for the first time that ATO could inhibit proliferation and executes apoptosis in NB-4 cells through trammeling NF-κB-mediated suppression of p73 transcription. These results are the ones to indicate that the inhibitory effect of ATO on NF-κB is through suppression of p65 phosphorylation and cramping the mRNA expression of IKK2, Nemo and ATM. Quelling NF-κB by ATO caused significant reduction in mRNA levels of its target genes including XIAP, BCL-XL, IAP1 and survivin. Our outcomes show for the first time that the preventive impacts of ATO on proliferation of NB-4 cells might be through transcriptionally stimulation of WNK2 and Lipocalin 2.

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Pharmacogenetic factors in metabolism, transport and toxicity of cytarabine treatment in patients with AML

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Background: Cytarabine is commonly used in the treatment of acute myeloid leukemia (AML). However, therapy associated side effects like neutropenia are often leading to severe infections, which are the most frequent cause for therapy associated mortality. Polymorphisms in genes encoding for cytarabine biotransformation or transport are considered to be an important factor contributing to individual drug toxicity in patients undergoing chemotherapy with cytarabine. For a suitable drug response cytarabine has to be actively transported into the cell via the human concentrative nucleoside transporter 1 (hCNT1) and the equilibrative nucleoside transporters 1 and 2 (hENT1, hENT2), activated by deoxycytidine kinase (dCK) and not extensively inactivated by cytidine deaminase (CDA).
Material and Methods: We analysed genetic polymorphisms in cytarabine biotransformation, transport and genes involved in cytarabine toxicity using PCR-RFLP and RealTime PCR in 322 adults younger than 60 years of age with cytogenetically normal AML. Patients participated in one of four clinical trials on drug therapy of AML. All trials used double-induction therapy with idarubicin, cytarabine and etoposide and several cycles of consolidation therapy with high-dose cytarabine. Cytarabine toxicity was measured as reconstitution time of total white blood cells (WBC) and neutrophils.
Results: The hCNT1 1561 C>T variant influenced the reconstitution time of WBC and neutrophils significantly ($p = 0.02$ and 0.03, respectively),