New drugs and mechanism

501 2-AMINOPHENOXAZINE-3-ONE SUPPRESSES THE TUMORIGENESIS OF ADULT T CELL LEUKEMIA CELL LINES IMPLANTED INTO NOD/SCID MICE

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Background: Adult T cell leukemia (ATL) is a malignant tumor of human CD4+ T cells infected with a human retrovirus, T-cell lymphotropic virus type I (HTLV-I), and the treatment of this malignancy is very difficult, in spite of the chemotherapeutic treatment and allogeneic hematopoietic stem cell transplantation. Since we found that 2-aminophenoxazine-3-one (Ptx-3) which exerts strong anticancer effects on various cancer cell lines induced apoptosis of HTLV-I positive leukemia cells, we examined whether Ptx-3 may suppress the tumorigenesis of this malignant tumor transplanted in NOD/SCID mice.

Materials and Methods: HUT-102 cells, a T-cell line established from a patient with adult T cell leukemia (ATL) and constitutively expresses the genes of a human retrovirus, T lymphotropic virus type-1 (HTLV-1) were used for the experiments. Ptx-3 was synthesized by the reactions of o-amino phenol with bovine red cells. For the in vitro experiments, HUT-102 cells were incubated with or without Ptx-3, for 72 h in a humidified incubator containing 5 % CO2 and 95% air at 37°C. For the in vivo experiments, HUT-102 cells were implanted subcutaneously at the back of NOD/SCID mice with or without Ptx-3.

Results: Ptx-3 (41 μM) greatly suppressed the cell growth, and increased population of the apoptotic cells in HUT-102 cells, by upregulating the expression of FAS 20 times the control, and activating caspase 8 and 9 in the cells. These results suggest that Ptx-3 exerts the proapoptotic effects against HUT-102 cells, through the activation of the extrinsic and intrinsic apoptotic pathways. When HUT-102 cells were implanted subcutaneously at the back of NOD/SCID mice, and the mice were treated with Ptx-3 (10 μg/kg), simultaneously with inoculation of the cells and once a week for 3 weeks, or on 1 day after the inoculation of the cells, and then was administered once daily, for 3 weeks, the growth of the cells was completely suppressed. These results indicate that Phx-3 inhibits tumorigenesis of HUT-102 cells implanted in mice.

Conclusion: Our present results suggest that Ptx-3 may be useful as a therapeutic agent for the treatment of patients with ATL as it is refractory to the current therapies.

502 SELECTIVE INHIBITION OF CHYMOTRYPSIN-LIKE ACTIVITY OF THE PROTEASOME LEADS TO ANTI-TUMOR EFFECT IN WALDENSTROM MACROGLOBULINEMIA, IN VITRO AND IN VIVO

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Introduction: Proteasome inhibition represents a valid therapeutic approach in several malignancies and its use has been validated in Waldenstrom’s macroglobulinemia (WM). Nevertheless, a significant fraction of patients relapse, or develop toxicity due to high toxicity in non-transformed cells. Therefore preclinical evaluation of new proteasome inhibitors with a more targeted inhibition of clonal cells is needed in order to increase efficacy and improve patient outcome. We evaluated the anti-tumor activity of carfilzomib, a new selective chymotrypsin-like (CT-L) proteasome inhibitor in WM, both in vitro and in vivo.

Materials and methods: Primary WM cells were obtained from bone marrow (BM) of WM patients (CD19+ microbead selection). WM and IgM secreting low-grade lymphoma cell lines were used. Level of imunoproteasome (26S) and constitutive proteasome (20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and apoptotic pathways were determined by Western Blot. Effect of Carfilzomib on paracrine synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and proteasome (c20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and apoptotic pathways were determined by Western Blot. Effect of Carfilzomib on paracrine synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and proteasome (c20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and apoptotic pathways were determined by Western Blot.

Results: Carfilzomib inhibited the CT-L activity of both i20S (LMP7) and c20S (L5) in primary WM cells, leading to inhibition of proliferation and induction of cytotoxicity; supported by increased PARP, caspase-9, -8 and -3 cleavage, as well as induced activation of c-jun-N-terminal kinase and ER-stress in a dose-dependent manner. Carfilzomib targeted WM cells even in the context of BM milieu, where inhibition of adhesion and migration were observed, together with induction of WM growth even in presence of BMSCs. Combination of carfilzomib and bortezomib induced synergistic cytotoxicity in WM cell lines, shown by enhanced PARP, caspase-9 and -3 cleavage, and synergy in inhibiting the CT-L activity of the i20S and c20S. Anti-tumor activity of carfilzomib has been validated in vivo, where carfilzomib-treated mice presented with a significant lower number of tumor cells (P<.05); increased percentage of apoptotic WM cells (P<.05); and reduced serum IgM levels (P<.05), as compared to control mice.

Conclusion: These findings suggest that targeting i20S and c20S CT-L activity by carfilzomib represents a valid anti-tumor strategy in WM, and in other IgM secreting lymphomas.

503 THERANOSTIC APPROACH FOR LYMPHOMA THERAPY USING PLATINUM-ETHYLENEDICISTEINE--NACETYLGLOUCOSAMINE (PT-ECG).

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Introduction: Aggressive Non-Hodgkin lymphomas (NHL), such as diffuse large B cell lymphoma (DLBCL) and mantle cell lymphoma (MCL), are very common in the US with increasing incidences. The emerging area in lymphoma biology involving energy metabolism has begun to identify likely potential molecular targets for novel therapeutics that can fundamentally change the conventional treatment of cancer. Glucose metabolism, besides its basic metabolic functions in cell physiology, has been shown to provide the major energy source fueling tumor cell growth and survival. We have developed a technology using ethylenedicysteine (EC) as a chelator to conjugate to glucosamine, creating a vehicle (EC-G) that can target highly proliferative cells. By conjugating a therapeutic metal Platinum to ECG (Pt-ECG), we have developed a therapeutic agent that target highly proliferative lymphoma cells.

Material and Methods: Pt-ECG was synthesized by mixing EC-G with K2PtCl4. Structure was confirmed by 1H-, Pt-NMR, elemental analysis and mass spectra. The purity of Pt-ECG was greater than 98.13%. Proliferation assays for Pt-ECG on 12 DLBCL cell lines and 10 MCL cell lines were performed using thymidine incorporation method. Apoptosis was detected using Annexin V assays. DNA damage was analyzed by detecting nuclear protein level of phospho-γH2A using Western blot and confocal microscopy techniques.

Results: Pt-ECG is highly effective in vitro, inhibiting lymphoma cell growth in low micromolar concentration (10-50 uM). Pt-ECG enters the nucleus, induces DNA damage by activating γH2AX, resulting in cell apoptosis.

Conclusion: These results suggest that the metallic pharmaceutical agent Pt-ECG is an excellent potential candidate for targeted therapy in aggressive lymphomas. This application further defines the term theranostic for personalized medicine strategies utilizing bifunctional imaging/therapeutic agents.

504 RECRUIT-TANDABS: ENGAGING IMMUNE CELLS TO KILL CANCER CELLS -- AFM13: A BISPECIFIC TETRAVALENT TANDAB FOR TREATING HODGKIN LYMPHOMA

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Introduction: AFM13 is a RECRUIT-TandAb® for the treatment of Hodgkin Lymphoma (HL) recruiting natural killer (NK) cells and macrophages to the specific CD30 surface antigen on HL cells. A similar approach using two other bispecifics showed promising results in clinical trials, as described by Hartmann et al (1997 and 2001) and Borghmann et al 2002. AFM13 is a bispecific, tetravalent human antibody comprising solely variable domains binding CD30 on HL cells and CD16a on NK-cells and macrophages. AFM13 thereby addresses key issues of monoclonal antibodies (both normal and ADCG enhanced), such as (i) the V/F polymorphism and (ii) the non-selective binding to immune effector cells using two Fv domains. RECRUIT-TandAbs (Abω) bind immune effector cells with high affinity right after infusion thereby targeting these cells to the tumor by binding to a tumor-associated antigen. AFM13 is in clinical trials in HL patients and, in the first dosing levels, appeared to be safe and well tolerated.

Method and Results: The anti-CD30 moiety of AFM13 was derived from a mAb that had already shown promising results in clinical trials. The human antibody specific for the CD16a receptor on NK cells was derived from Affirmed’s human phage display antibody libraries. The heavy and light chain variable domains of both antibodies were engineered into a four-domain TandAb® gene product. The linkers between the domains were designed to prevent intra-domain pairing and to force dimerization resulting in the formation of the functional homodimeric TandAb® molecule. AFM13 is able to rapidly induce the lysis of CD30+ cells at picomolar concentrations in presence of PBMCs. Intensive in vitro characterization of AFM13 has demonstrated its remarkable specificity for just the CD16a receptor on NK cells, which, however, only become activated in the presence of tumor cells. There is no systemic activation of NK-
cells. A robust and scalable GMP production in mammalian cells, a down-stream program in cynomolgus monkeys was successfully completed. In 2010 AFM13 entered a dose escalating phase I clinical trial. Several patients have been dosed so far and AFM13 appeared to be safe and well tolerated.

Conclusions:
AFM13 was well tolerated in toxicology studies in Cynomolgus monkeys. A robust and scalable GMP production in mammalian cells, a down-stream program in cynomolgus monkeys was successfully completed. In 2010 AFM13 entered a dose escalating phase I clinical trial. Several patients have been dosed so far and AFM13 appeared to be safe and well tolerated.

505 ALTERED CD20 CONFORMATION AT THE CELL SURFACE OF RITUXIMAB REFRACTORY TUMOR CELLS – A NOVEL MECHANISM OF RITUXIMAB RESISTANCE

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Introduction:
The anti-CD20 monoclonal antibody (mAb), rituximab, in combination with chemotherapy (R-CHOP) is widely used for the treatment of B-cell malignancies. However, a sizable proportion of patients subsequently develop resistance. Although currently obscure, the elucidation of the mechanisms underlying this resistance are clearly vital in this post-rituximab era.

Patients and Methods:
Tumor samples from a follicular lymphoma patient who developed resistance to R-CHOP immunochemistry after initial complete response were examined in a selection of in vitro assays. CD20 expression was confirmed by immunohistochemical staining, flow cytometry, and immunoblotting. Lipid raft re-distribution of CD20, alongside CDC and ADCC activity were examined as previously described (Cragg et al. Blood 2003; Hiraga et al. Blood 2009).

Results:
Pathological specimens taken from patient lymph node samples were consistently CD20 (L26) positive during the whole clinical course irrespective of resistance to immunochemistry, a finding confirmed by routine flow cytometric analyses using the B1 mAb. However, rituximab binding to tumor cells obtained at the refractory stage was weaker than expected and resulted in resistance in ADCC and CDC assays (52% specific lysis of Dauchi cells vs 6% tumor in ADCC; 39% vs 9%, respectively in CDC). More comprehensive flow cytometry analyses using a panel of anti-CD20 mAb revealed weak staining with rituximab, ofatumumab and GA101, but strong binding with B1. Importantly, no mutation in the coding sequence of the MS4A1 gene was observed and CD20 in the refractory tumor cells was predominantly phosphorylated, indicating correct intracellular folding. Lipid raft distribution analysis, revealed the surprising finding that B1 efficiently re-distributed CD20 into lipid rafts whilst rituximab and ofatumumab did not.

Conclusion:
These data, albeit derived from a single patient, indicate that tumor cells may become resistant to R-CHOP treatment due to a modification in the distribution of CD20 in the plasma membrane, resulting in impaired binding and altered lipid raft redistribution, concordant with a reduction in CDC and ADCC. Further investigation into the frequency and underlying biology of this novel resistance mechanism is required.

506 THE TYPE II CD20 ANTIBODY GA101 DIFFERS FROM THE TYPE I CD20 ANTIBODY RITUXIMAB IN INDUCING DIFFERENTIAL GENE EXPRESSION PROFILES AND BY BINDING SEPARATE AND FUNCTIONALLY DISTINCT CD20 COMPLEXES ON B CELL LYMPHOMA CELL LINES

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Rituximab is a type I CD20 antibody, whereas GA101 is a type II, glycoengineered CD20 antibody currently in PhIII clinical trials. Their MOAs differ as a result of different cell-autonomous effects and different immune effector functions. While rituximab efficiently mediates CDC, GA101 induces enhanced direct cell death and ADCC. To investigate the different direct effects, we analyzed gene expression profiles of lymphoma cells after antibody treatment. Both, GA101 and rituximab, rapidly trigger gene expression changes. A subset of the regulated genes are targets of BCR activation (EGR2, BCL2A1, RGS1, NAB2). Not surprisingly, the signaling capacity of CD20/antibody complexes depends on the cellular background, since cell lines representing different B-cell stages responded differently. For instance, SUDHL4 DLBCL cells responded strongly to rituximab treatment, while Z138 MCL cells showed less pronounced gene expression changes. Interestingly, Z138 responded more pronounced to GA101 than to rituximab, while the reverse was true for SUDHL4. Principal component analyses indicates that, apart from cell background, the antibody type is the strongest determinant. For a given cell line, the number of genes regulated and the amplitude of changes differ not only for rituximab and GA101, but in a similar manner for other type I (LT20G7, LEN97) and type II (BML90B1, BH20) antibodies. Unbiased hierarchical clustering analysis of gene expression changes in SUDHL4 discriminated type I from type II antibodies suggesting that CD20 complexes recognized by type I or II antibodies have inherently different signaling capacities. Next we investigated cell surface compartmentalization of CD20 subpopulations recognized by the different antibodies by confocal microscopy. Rituximab and GA101 labeled with different fluorophores showed little colocalization on lymphoma cells (Z138, Ramos) and to some extent also on normal B-cells. Time-lapse microscopy of Z138 cells revealed that GA101/ CD20 complexes on the cell surface were very static and strongly accumulated at cell-cell contact sites, whereas rituximab/CD20 complexes were highly dynamic at non-contact sites. They only occasionally passed through sites of cell-cell contact. In summary, gene expression and membrane compartmentalization data imply that type I and II antibodies bind functionally distinct CD20 subpopulations.

507 BENZAMUSTINE AND LENALIDOMIDE IN RELAPSED/REFRACTORY LYMPHOMA MALIGNANCIES

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Background: Standard treatment for B-cell NHL includes Rituximab (R) and conventional chemotherapy. While ORRs with these regimens are roughly 80-90%, the CR rates are relatively lower and pts often relapse. Bendamustine (L) and Lenalidomide (L) have both shown efficacy as single-agents in relapsed and refractory lymphoid malignancies. The combination of these two agents, however, has not yet been evaluated. Therefore, we proposed a 2-stage phase I study in which B and L were combined in relapsed/refractory lymphoid malignancies (Stage I) in order to determine the MTD of L for future combination of BL in relapsed/refractory B-cell malignancies (Stage II). We report the preliminary results of Stage I here.

Methods:
Pts with relapsed/refractory NHL or Hodgkin’s lymphoma (HL) and adequate performance status and organ function were eligible. This phase I study utilized a standard 3+3 dose-escalation design. In Stage I all pts received B 90 mg/m2 IV on D1, 2 with escalating doses of L (5, 10, 15, and 20 mg) po daily for each 28-day cycle. Pts received 6 cycles of BL followed by 6 cycles of L as long as tolerated until disease progression.

Results:
Seven pts have been enrolled on study thus far. The median age was 52 years, 43% were male, and all were Ann Arbor Stage III/IV. Specific histologies included DLBCL (3), HL (2), follicular lymphoma with transformation (1), and cutaneous T-cell lymphoma (1). An MTD has not yet been reached for L. Of the first two pts on study who received L 5 mg daily, the second pt had a DLT of grade 3 rash. Therefore, the dose of L was de-escalated to 5 mg every other day with the third pt. As rash is a common toxicity with L, the protocol was subsequently amended so that only grade 4 rash or grade 3 rash that did not resolve to < grade 2 within 10 days despite steroids were to be considered DLTs. In addition, L was to be given only 21 days of each cycle. The trial was reinstituted after these amendments, and 3 new pts were enrolled into Cohort 1 of L 5 mg daily. The first pt in this new cohort died during cycle 1 due to progressive disease and was replaced by another pt. There were no further DLTs or grade 3/4 rashes found. Common grade 3/4 toxicities included anemia (57%), leucopenia (57%), neutropenia (43%). Pts completed a median of 2 cycles of therapy. Of the 7 pts, 6 progressed on BL. One pt with DLBCL achieved a CR within 2 months and proceeded to transplant. The most common reason for discontinuing treatment was progressive disease. Cohort 1 has been completed. Enrollment to Cohort 2 began in January 2011.

Conclusion:
BL appears to be tolerable in relapsed/refractory, advanced lymphoid malignancies. Preliminary evidence suggests limited efficacy in this highly refractory population. Additional pts are being accrued to determine the MTD of L prior to adding R in those with a B-cell NHL.