9201 ORAL

Complete Remissions Observed in a Subset of Pediatric Patients With CD30-expressing Malignant Lymphomas Treated in Clinical Studies of Brentuximab Vedotin (SGN-35)

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Background: Brentuximab vedotin (SGN-35) consists of an anti-CD30 antibody conjugated by a plasma-stable linker to the potent antimicrotubule agent, monomethyl auristatin E (MMAE). Brentuximab vedotin selectively induces apoptotic death of CD30-expressing cells by binding to cells, followed by internalization and release of MMAE. Brentuximab vedotin has shown encouraging anti-tumour activity in adult patients with relapsed or refractory Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL).
Methods: A subset of pediatric patients (12−17 yrs) was enrolled in two phase 1 and two phase 2 multicenter studies. Patients with CD30-expressing hematologic malignancies received intravenous brentuximab vedotin as a 30-minute outpatient infusion in weekly treatment cycles (3 out of 4 weeks) at doses of 0.8 or 1.2 mg/kg (1 pt each) or every 3 weeks at doses of 1.2 mg/kg (1 pt) or 1.8 mg/kg (6 pts). Informed consent and assent were obtained for all patients. Clinical response was assessed by the investigator using the Revised Response Criteria for Malignant Lymphoma (Cheson 2007).
Results: Across the 4 studies, 9 pediatric patients were enrolled, ranging in age from 12−17 yrs. Diagnosis was HL for 5 patients and sALCL for 4 patients. Baseline Karnofsky performance was 0 (5 pts) or 1 (4 pts). The 5 male and 4 female patients all had at least 1 prior systemic chemotherapy regimen (range, 2−7) and 4 patients had prior autologous stem cell transplantation. Patients received 3−16+ cycles of brentuximab vedotin. The most frequent treatment-emergent adverse events (TEAEs) were fatigue, nausea, and peripheral neuropathy. Treatment-related Grade 1−2 peripheral neuropathy was reported in 7 patients. Three of 9 patients experienced related TEAEs $\geqslant$Grade 3 (hyperesthesia, leukocytopenia, and neutropenia). Six of 9 patients obtained complete remission (CR; 2/5 HL pts and 4/4 sALCL pts) and the remaining 3 HL patients achieved best response of stable disease. Duration of CR to date has been approximately 3−12 months and 5/6 patients remain in CR after 6−12+ months of follow up.
Conclusions: Consistent with findings in the adult population, treatment with brentuximab vedotin induced CR in 6 of 9 pediatric patients with relapsed/refractory HL or sALCL, of which 5/6 patients remain in CR, and adverse events were manageable. HL and sALCL are relatively common pediatric lymphomas and further study in children and young adults is planned.

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MRC Myeloma IX Trial − Characterizing the Biologic and Temporal Basis of Efficacy of Long-term Bisphosphonate Treatment in Patients With Multiple Myeloma

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Background: Myeloma IX examined the effect of thalidomide-based induction and maintenance and 2 different bisphosphonates (BPs), intravenous zoledronic acid (ZOL) and oral clodronate (CLO), in patients with multiple myeloma (MM). In 1960 evaluable patients, ZOL improved overall survival (OS) by 5.5 mo ($P = 0.04$) and significantly reduced the risk of skeletal-related events (SREs) vs CLO (Morgan et al. *Lancet*. 2010). However, there has been little previous investigation into the effects of BPs by MM prognostic variables and treatment duration.
Methods: Patients with newly diagnosed MM were assigned to intensive or non-intensive treatment pathways and then randomized to concurrent ZOL or CLO, each of which was continued at least until disease progression. The effects of ZOL vs CLO on OS were assessed in subgroup analyses (demographics, biologic disease characteristics, and duration of BP therapy). Baseline bone disease (BD+ or BD-) was screened using axial skeletal surveys. FISH analysis was performed using standard methodology in a substudy ($n \approx 1000$).
Results: In subgroup analyses, ZOL OS benefit vs CLO was not affected by sex, ISS stage, or specific myeloma genotypes. BD+ patients had significantly shorter OS (median = 45.5 mo, n = 1401) vs BD- patients (median = 51.6 mo, n = 540; $P = 0.009$). OS benefit with ZOL vs CLO was most profound in BD+ patients (n = 1401; ~71% of total) and in patients receiving BPs for $\geqslant$ 2 years (n = 582; ~30% of total). In the latter subset, ZOL improved OS vs CLO from randomization (median not reached for either; log-rank $P = 0.02$) and from first on-study disease progression (median = 34 mo [ZOL] vs 27 mo [CLO]; log-rank $P = 0.03$). Additional efficacy and tolerability analyses are ongoing in this subset.
Conclusions: OS benefits with ZOL vs CLO are independent of demographic and baseline disease characteristics evaluated except for BD (benefit restricted to BD+ patients). OS improvements with ZOL were most profound in patients receiving BPs for $\geqslant$ 2 years, and suggest continued benefits despite disease progression.

9203 ORAL

Targeting Pro-survival Bcl-2 Proteins in Haematological Malignancies Driven by Activated Mutant JAK2

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Background: Oncogenic mutations and translocations of JAK2 are frequently detected in myelo- and lymphoproliferative disorders. One example is the fusion of the transcription factor TEL (ETV6) to the kinase domain of JAK2 identified in human T cell acute lymphocytic leukemia (ALL). Furthermore, different activating point mutations of JAK2 have recently been identified in pediatric acute pre-B cell lymphoblastic leukemias (pre B-ALL). Using a TEL-JAK2-driven mouse model of T-ALL, this project aims to identify the critical downstream effectors of oncogenic JAK2 signalling to discover novel strategies to treat JAK2-driven malignancies.
Material and Methods: EμTEL-JAK2-transgenic mice develop a rapid onset T-ALL which is transplantable into C57Bl/6 mice. Using Western Blot, FACS analysis and cell death assays we studied molecular pathways and survival in EμTEL-JAK2 T-ALL cells *ex vivo*. Furthermore, we analysed the therapeutic efficacy of the JAK2 inhibitor TG101209, the BH3-mimetic ABT-737, the chemotherapeutic drugs Etoposide and Cyclophosphamide, or combinations of these drugs in cohorts of mice with secondary EμTEL-JAK2 T-ALL.
Results: When cultured *ex vivo*, EμTEL-JAK2 T cell leukemic cells exhibited resistance to common chemotherapeutics, but were sensitive to the JAK2 inhibitor TG101209. Consistent with our understanding that chemotherapeutic drugs induce tumour cell apoptosis via the intrinsic apoptotic pathway, we demonstrated that these cells express high levels of anti-apoptotic Bcl-2 and Bcl-xL. When treated with the Bcl-2/Bcl-xL antagonist ABT-737, EμTEL-JAK2 T-ALL cells underwent rapid apoptosis. Moreover, mice bearing secondary EμTEL-JAK2 leukemias displayed prolonged survival following treatment with ABT-737 or TG101209 concomitant with robust tumour cell apoptosis *in vivo*. Furthermore, combination therapies involving ABT-737, TG101209 and Etoposide or Cyclophosphamide eradicated disease.
Conclusions: We hypothesise that TEL-JAK2 expression induces Bcl-2/Bcl-xL expression through constitutive activation of STAT5, and posit that Bcl-2/Bcl-xL upregulation may be a common feature of JAK2-driven haematological malignancies. Therapeutic regimens using ABT-737 in combination with JAK2 inhibitors or standard chemotherapeutic drugs may therefore be a rational approach to treating these diseases. We are currently testing this hypothesis in a xenotransplantation model using human pre-B ALL cells expressing constitutively active mutant JAK2.

9204 ORAL

Telomerase Inhibition Affects Fludarabine Sensitivity in Primary Chronic Lymphocytic Leukemia Lymphocytes

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Background: B-cell chronic lymphocytic leukemia (CLL) is clinically very heterogeneous and has a highly variable clinical course. Different studies have demonstrated that telomere length and telomerase activity are good prognostic factors in CLL. These studies have prompted the assessment of Imetelstat, a telomerase inhibitor which targets the telomerase RNA component hTR, and is currently in phase I-II clinical trial in CLL as a single agent.

Methods: DNA is extracted from lymphocytes isolated from leukemia patient blood. MTT assay is used to assess cytotoxicity. Protein levels are assessed by Western blot analysis. Telomere length is assessed using qPCR. Telomerase activity is assessed using the Telomeric Repeat Amplification Protocol kit by Roche. DNA-PK autophosphorylation is determined by FACS analysis.
Results: We report that telomerase activity was present in 48% of lymphocyte samples from 24 CLL patients and that treatment with Imetelstat alone did not affect the survival of primary CLL lymphocytes *in vitro*. Nonetheless, Imetelsat increased the sensitivity of lymphocytes from CLL patients to fludarabine, independently of basal telomerase activity. Imetelstat inhibited fludarabine-induced DNA-PK autophosphorylation, a surrogate marker of DNA-PK activity, in CLL lymphocytes, to the same extent than the DNA-PK inhibitor NU7026. The effect of Imetelstat on fludarabine sensitivity was associated with a lower basal protein expression of the DNA binding subunit of DNA-PK, Ku80.
Conclusion: Our results suggest that Imetelstat can inhibit fludarabine-induced DNA-PK activity in primary CLL lymphocytes. We speculate that there may be a functional interaction between hTR and DNA-PK in primary CLL lymphocytes and conclude that Imetelstat in combination with fludarabine may be useful to decrease the tumour burden in CLL.

9205 ORAL

Mammalian Target of Rapamycin (mTOR) Activity Dependent Protein Expression and Rapamycin Sensitivity in Pediatric Acute Lymphoblastic Leukemias

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Deregulation of signal transduction pathways could be a key event in leukemogenesis. mTOR complexes with different rapamycin sensitivity are central mediators of several signaling pathways, regulate cell proliferation, survival and protein translation. The mTOR pathway has recently attracted a lot of attention as a potential target in oncological therapy, including hematological diseases. However, limited data exists about the activity of mTOR in lymphoid malignancies, especially in pediatric acute lymphoblastic leukemia (ALL), representing nearly one third of all pediatric cancers.
We characterized the expression of mTOR activity dependent phospho-proteins by ELISA (pmTOR, p4EBP1, pS6) in human leukemia/lymphoma cell lines, isolated peripheral mononuclear cells, T- and B-cells, and lymphoblasts from childhood ALL patients. Expression was measured before and during therapy and at relapses during a minimum of a 2-year follow-up of 22 patients. Phospho-protein levels and clinical data were statistically evaluated. The effect of rapamycin treatment on apoptosis and the amount of mTORC1/C2 complexes was measured by flow cytometry and immunocytochemistry in cell lines and short-term cultures.
Cell lines exhibited increased pS6 (2.4−8-fold) and p4EBP1 (62.5–72.5-fold) protein levels. Statistical analysis of more than 80 ALL samples and non-leukemic bone marrow/blood samples showed that p4EBP1 expression was significantly higher (20−58-fold) in ALL samples at diagnosis; the decrease of p4EBP1 expression followed the effectivity of chemotherapy in all patients. We also found that mTOR activity was significantly higher at diagnosis in the samples of patients with worse prognosis, both in B- and T-ALL; p4EBP1 expression was retained and increased above day 0 level at relapses. In vitro rapamycin treatment induced apoptosis in cell lines and in short-term ALL cultures only when p4EBP1 expression concomitantly decreased. In vitro results also suggest that Rictor/Raptor expression correlates with rapamycin resistance in lymphoma/leukemia cells.
Our results suggest that mTOR activity is elevated in ALL cells, which can be monitored by measuring p4EBP1 by ELISA. p4EBP1 may be an important marker for identifying patients with poor prognosis at diagnosis, following p4EBP1 expression could help in the earlier detection of relapse during the therapy; and it may also be useful for the selection of patients who may benefit from rapalog tratment.
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Poster Presentations (Sun, 25 Sep, 14:00−16:30)
Haematological Malignancies and Myeloma

9206 POSTER

Redox-sensitive P73-related Pro-apoptotic Effect of the Polyphenolic-rich Aronia Melanocarpa Juice on Human Acute Lymphoblastic Leukemia Cells

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Background: Natural products derived from plants have received considerable attention as potential cancer chemopreventive and chemotherapeutic agents over few decades. On the basis of epidemiological and animal studies, it has been recurrently reported that diets rich in fruits and vegetables are associated with a reduced rate of cancer mortality. Natural products rich in polyphenols have been shown to have strong chemopreventive properties in different types of cancer cells. Moreover, the polyphenol-induced cytotoxic effect appears to target specifically cancer cells.
Aronia melanocarpa also known as black chokeberry is a shrub native from North America. *Aronia melanocarpa* juice (AMJ) is one of the richest sources of natural polyphenols. AMJ has been shown to have numerous health benefits, including cardioprotective, hepatoprotective and antidiabetic activities. Several *in vitro* and *in vivo* studies indicate that *Aronia melanocarpa* extracts have also antiproliferative effects against colon cancer cells. The aim of the present study was to determine whether AMJ inhibits proliferation of the human acute lymphoblastic leukaemia cells, and if so, to identify the underlying molecular mechanism in particular the role of reactive oxygen species (ROS).
Material and Methods: Human acute lymphoblastic leukemia Jurkat cell line, human primary lymphoblastic leukemia cells and normal human primary T-lymphocytes were used in the study. MTS assay, Cell cycle phase distribution and Apoptosis analysis were performed to study the effect of AMI on proliferation, cell cycle and apoptosis respectively. The formation of ROS was determined by staining with dihydroethidine (DHE). Western blot experiments were performed to detect p73, Cytochrome c, Cyclin B1, Caspase 3 and UHRF1 in Jurkat cells.
Results: We have found that AMJ inhibited cell proliferation and induced cell cycle arrest in G_2/M phase leading to apoptotis. These effects are associated with an upregulation of the tumour suppressor p73 and cleaved caspase 3, and a downregulation of cyclin B1 and UHRF1. AMJ significantly increased the formation of ROS associated with the release of cytochrome c into the cytoplasm. Treatment with intracellular ROS scavengers prevented AMJ-induced apoptosis and upregulation of p73 and active caspase 3 expression. Moreover, it was found that AMI selectively killed the primary lymphoblastic leukemia cells without effecting normal human primary T-lymphocytes.
Conclusion: These findings indicate that AMJ exhibits potent anticancer activity through a redox-sensitive mechanism in the p53-deficient Jurkat cells. In addition, AMJ exerted a strong pro-apoptotic effect in human primary lymphoblastic leukemia cells but not in human normal primary T-lymphocytes. In conclusion, these results suggest that AMJ has chemo-preventive and chemotherapeutic properties against acute lymphoblastic leukemia by selectively targeting lymphoblast-derived tumour cells.

9207 POSTER

Evaluation of Effect of Caffeic Acid Phenyl Ester on Acute T-Lymphoblastic Leukemia Cells by Mitochondria and Peroxisome

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Background: More than 70% of chemotherapeutic agents currently used for treatment of leukemia have been derived from natural sources. Leukemia mainly can be classified as acute and chronic and for the acute leukemia treated with current agents, 5 years survival rates have been around 40−50%. There have been new therapy models for specific targets in the last decade. New targeted drugs have been mostly combined with classical drugs. But, classical chemotherapy agents are still the main treatment for leukemia. Complications associated with classical drugs have brought forth new researches to develop new cancer treatment agents. Caffeic acid phenethyl ester (CAPE) is the active compound that has wide spectrum effects such as antioxidant, anti-inflammatory, anti-viral, carcinogenetic and anti-cancer. PPAR-gamma plays a key role in atherosclerosis, inflammation, obesity, diabetes, immune response and