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Treatment of new cases of acute promyelocytic leukaemia by arsenic trioxide

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Purpose: Arsenic Trioxide approved for treatment of relapsed or refractory APL to ATRA. We studied the effects of Arsenic Trioxide as first line treatment of new cases of APL and their follow up.
Material and Methods: we studied 63 new cases of APL diagnosed by morphologic criteria and confirmed by cytogenetic, RT-PCR for PML/RARA and/or FISH.
Our patients were 28 males and35 females with median age 27±11.98. Patients treated by infusion of 0.15mg/kg/d of Arsenic Trioxide to complete remission by morphologic criteria or till day +60. In patients who complete remission achieved, after 28 days rest, again we began Arsenic Trioxide 0.15mg/kg/d for 28 days as consolidation.
Results: complete remission were achieved in 57 patients(90.5%) and 6 early mortality. Median time to complete remission was 30+/-6.6 days. Most common cause of mortality was APL maturation syndrome (4 cases)
Most common toxicities during induction phase were, APL maturation syndrome (14.7%), serositis(11.4%) and hepatotoxicity (18%).
88.5% of patients are alive with a mediam follow up of 12+/-10.02 months. 11 relapses observed in our patients and complete remission achieved with retreatment by Arsenic trioxide in 8 of them.
Mean survival time of patients by Kaplan-Meier method was 33.91 months (CI95% 30.98–37). Most common cause of death were APL maturation syndrome in 3 patients and relapse in 3 cases.
Conclusion: Arsenic Trioxide is acceptable as first line treatment of APL and its result is comparable to ATRA with chemotherapy.

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Nuclear survivin is a powerful novel prognostic marker in gastroenteropancreatic neuroendocrine tumour disease

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Background: Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) represent a rare and rather heterogeneous tumour entity. The growth pattern of GEP-NETs ranges from very slowly to fast growing, aggressive types of tumours. Survivin, a member of the family of apoptosis inhibitors, is a bifunctional protein that suppresses apoptosis and regulates cell division.
Aims: Here we determined the prognostic value of survivin in a series of GEP-NETs.
Patients and Methods: Tumour specimens from 104 patients (38 foregut, 53 midgut, 13 hindgut NETs) were studied immunohistochemically for cytoplasmic and nuclear survivin expression as well as for ki-67 antigen expression. 5-year-follow-up was complete in 89 patients. 29 patients with non-metastatic, well-differentiated GEP-NETs had been curatively treated by surgical or endoscopic tumour resection; therefore they were excluded from statistical analysis of survival. Kaplan-Meier-survival curves were calculated for 60 patients with advanced metastatic GEP-NETs.
Results: No recurrencies or tumour-associated deaths occurred in the 29 patients with localised well-differentiated GEP-NETs. All tumours of this group were negative for nuclear survivin. In the 60 patients with advanced metastatic GEP-NETs 15/60 (25%) tumours were nuclear survivin positive. Those 15 patients had a statistically significant worse prognosis (survival of 8 versus 115 months, $p<0.00001$). Nuclear survivin expression was strongly correlated with the differentiation grade of the tumour: Only 3/47 well-differentiated tumours displayed nuclear survivin, but 12/13 undifferentiated tumours did so.
Conclusions: Nuclear survivin expression appears to be upregulated during progression of GEP-NETs. The analysis of nuclear survivin expression identifies subgroups in metastatic GEP-NETs with good (survivin-) or with less favorable prognosis (survivin+). We propose that the determination of nuclear survivin expression could be used to individualize therapeutic strategies in GEP-NETs in the future.

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A phase I and pharmacokinetic (PK) study of an agonistic, fully human monoclonal antibody, HGS-ETR2, to the TNF-alpha related apoptosis inducing ligand receptor 2 (TRAIL R2) in patients with advanced cancer

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Introduction: A primary goal of cancer therapy is the selective induction of apoptosis in tumour cells. TRAIL induces apoptosis in a wide variety of cancer cells, whilst sparing most normal cells, by activating its death receptors TRAIL R1 (DR4) and TRAIL R2 (DR5) and downstream caspases. HGS-ETR2 is a high-affinity, recombinant fully human, $IgG_{1\lambda}$ monoclonal antibody (mAb) agonistic and specific to TRAIL R2. It induces apoptosis in TRAIL R2-expressing human tumour cell lines and tumour regression in established xenografts.
Methods: HGS-ETR2 was administered by IV infusion every 3 weeks at 0.1, 0.3 (4 pts each; 30 minute infusion) and 1 mg/Kg (6 pts; 2 hour infusion).
Results: Fourteen patients (age range: 25–70 years; 11 males) have received a total of 34 doses (range: 1 to 8 doses per patient) of HGS-ETR2. A patient with rapidly progressing metastatic chondrosarcoma has had continuing disease stabilization, receiving 8 courses of HGS-ETR2 to date. HGS-ETR2 has been well tolerated with minimal toxicity. One patient who received 1 mg/Kg developed CTCAE grade 3 asymptomatic rise in his serum amylase, detected on day 15 of course 1, resolving to grade 2 on day 23 and to baseline by day 43. This may have been related to the concurrent administration of ciprofloxacin. A further five patients were treated at this dose level without dose limiting toxicity or serious adverse events. Preliminary pharmacokinetic results are consistent with a two-compartment model with first-order elimination from the central compartment. At 0.3 mg/kg, the $t_{1/2\beta}$ranged from 10.03 to 14.98 days with a clearance that ranged from 4.28 to 4.83 mL/day/kg. The volume of distribution at steady state ranged from 65 to 88 mL/Kg and is 1.6 fold larger than the volume of distribution of the central compartment, indicating that HGS-ETR2 distributes to tissues. No HAHA antibodies have been detected thus far.
Conclusion: HGS-ETR2 administration is well tolerated and further dose escalation is anticipated.

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BARD1 required for telomere maintenance and control of genomic stability

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BARD1, the major protein binding partner of BRCA1, acts together with BRCA1 in repair functions upon genotoxic stress and ubiquitination. Mutations in BARD1 or BRCA1 predispose to cancer of the breast and ovary. A BRCA1-independent function of BARD1 was discovered in signalling from genotoxic stress to apoptosis. Upregulation of BARD1 in vivo, as observed upon stress, or overexpression in vitro lead to stabilization of p53 and induction of apoptosis. Repression of BARD1, by stable expression of BARD1 antisense RNA, results in genetic instability and resistance to apoptosis inducing drugs (Irminger-Finger et al., JCB 1998; Mol Cell 2001). Since BARD1 is upregulated upon genotoxic stress in vitro and in vivo, and triggers apoptosis by binding and stabilizing p53, it acts as critical messenger between genotoxic stress and apoptosis.
Mice deficient for telomerase show increased levels of apoptosis in a number of tissues but also increased incidence of tumorigenesis associated with genomic instability due to telomere attrition. We hypothesized that BARD1 might be involved in signalling from critically short telomeres towards apoptosis, since BARD1 repression or deficiency causes a premalignant phenotype (Irminger-Finger et al., 1998) and genomic instability (Irminger-Finger et al., 1998; Joukov et al., 2001; McCarthy et al., MCB, 2003).
To investigate this issue, telomere length was measured in BARD1-repressed or deficient cells by FISH and flow-FISH. We demonstrate that BARD1 repression is associated with a high degree of genetic instability and aneuploidy, and chromosomal aberrations, due to telomeric fusions. We further show that cell lines with stable repression of BARD1, TAC-2/ABI (Irminger-Finger et al., JCB 1998), and ovarian cancer cells missing functional BARD1, NuTu-19, have short telomeres, genomic instability, and

activated telomerease, as shown by immuno-detection of telomerase and TRAP assays. Furthermore, inhibition of BARD1 expression by siRNA transfection leads to loss of telomeric sequences, telomeric fusions, and chromosomal translocations in mouse mammary gland cells TAC-2, primary rat mesothelial cells, and Hela, or MCF-7 cells. This indicates that BARD1 controls telomere structures independently of telomerase activity. A fraction of BARD1 co-localizes with telomeres, as well as with the bona fide telomere binding protein TRF2, shown by FISH and immuno-staining and DNA chip assay. Our data are consistent with the following model: i) BARD1 might be sequestered on telomeres, ii) critically short telomeres would lead to an increase of unbound BARD1, iii) free BARD1 could act in apoptosis induction by binding and stabilizing p53 as demonstrated previously (Irminger-Finger Mol Cell 2001). Repression of BARD1 allows cells with critically short telomeres continue to divide. Due to genomic instability, eventually, some of these cells can activate telomerase and therefore pass crisis.

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Both RIP and c-FLIP are required for inhibition of caspase-8 cleavage in TRAIL-DISC in human cancer cells

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Background: Many cancer cells express the death receptors DR4 and DR5 for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) yet are resistant to TRAIL-induced apoptosis. TRAIL induces apoptosis through the recruitment of Fas-associated death domain (FADD) and caspase-8 to DR4/DR5, leading to assembly of death-inducing signaling complex (DISC) where caspase-8 is cleaved and initiates apoptosis. To explore the mechanisms in TRAIL resistance, we analyzed TRAIL-DISC in resistant cancer cells.
Methods: Non-small cell lung carcinoma A549, H596 and H1792, colon carcinoma Caco-2 and Colo320, breast carcinoma MB456, and pancreatic carcinoma Panc-1 cell lines were included in the study. TRAIL-DISC was immunoprecipitated using Flag-tagged TRAIL and anti-Flag M2 antibody and examined on Western blots. Cell death was analyzed by acid phosphatase assay and caspase cleavage was examined on Western blots. Synthetic small interfering RNA (siRNA) was generated by Qiagen Inc. and transfected with TransMessenger transfection reagent.
Results: Receptor-interacting protein (RIP) and cellular Fas-associate death domain-like, IL-1β-converting enzyme-inhibitory protein (c-FLIP) were reported in TRAIL-DISC. Western blot analysis of TRAIL-DISC revealed RIP and c-FLIP in TRAIL resistant A549 and H596, but not sensitive H1792 cells. Western blots detected cleavage products of caspase-8, caspase-3 and DNA fragmentation factor 45 (DFF45) in the cytoplasm in the sensitive, but not in the resistant cells. Transfection of siRNA targeting *c-FLIP* gene inhibits c-FLIP expression and sensitized the resistant cells to TRAIL-induced apoptosis through caspase-8-mediated caspase cascade. The results suggested that c-FLIP inhibited caspase-8 cleavage in the DISC in the resistant cells. Detection of RIP-mediated inhibitor of κB kinase (IKK) complex in the DISC suggested that RIP mediated IKK-mediated nuclear factor-κB (NF-κB) activation in resistant cells. Transfection of siRNA specific to *RIP* gene, however, sensitized the resistant cells to TRAIL-induced apoptosis through caspase-8-mediated caspase cascade. Transfection of either RIP or c-FLIP siRNA in TRAIL resistant Caco-2, Colo320, MB456 and Panc-1 cell lines resulted in the cell sensitivity to TRAIL-induced apoptosis.
Conclusion: The results indicate that both RIP and c-FLIP are required for inhibition of caspase-8 cleavage in TRAIL-DISC and thus targeting either RIP or c-FLIP may provide novel therapeutic strategies in cancer therapies.

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Mutant p53 reactivation by PRIMA-1: a novel strategy for cancer therapy

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Mutant p53 reactivation in tumors should trigger massive apoptosis and thus eliminate the tumor. We previously identified PRIMA-1, a low molecular weight compound that reactivates mutant p53 and induces robust apoptosis in human tumor cells. Intravenous adminstration of PRIMA-1 inhibited human tumor xenograft growth in mice (1). These results were corroborated by our statistical analysis of available information in the National Cancer Institute database (2). Growth inhibition profiles for PRIMA-1 and known anticancer agents were analyzed. PRIMA-1 was consistently more efficient in inhibiting growth of mutant p53-carrying tumor cell lines compared to wild type p53-carrying lines, and sensitivity to PRIMA-1 was correlated to mutant p53 expression levels. This distinguishes PRIMA-1 from most known anticancer drugs which preferentially affect tumor cells carrying wild type p53. We have found that PRIMA-1 acts synergistically with several anticancer drugs to inhibit tumor cell growth. Our further studies revealed that PRIMA-1 and cisplatin showed synergistic induction of apoptosis in a mutant p53-dependent manner. Combined systemic treatment with low doses of PRIMA-1 and cisplatin produced a significant synergistic antitumor effect in mice carrying human tumor xenografts. Enhancement of mutant p53 expression levels by DNA-damaging chemotherapeutic drugs may increase sensitivity to PRIMA-1-induced apoptosis. The anticancer efficacy of PRIMA-1 will be tested in clinical trials. Reactivation of mutant p53 by PRIMA-1 alone or in combination with conventional chemotherapeutic drugs is a novel strategy for cancer therapy that should allow efficient elimination of mutant p53-carrying tumors.

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Analysis of survivin splice variant transcripts in human breast tumor cells

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Background: Survivin, is a member of the apoptosis inhibitors family, and is expressed in several human tumors. Two alternative splice variants of survivin (survivin-DEx3, and survivin-2B) differing in their anti-apoptotic properties were recently identified. While the anti-apoptotic effect of survivin-DEx3 is preserved, survivin-2B has lost its anti-apoptotic potential and may act as a naturally occurring antagonist of survivin and survivin-DEx3. Because in vivo studies have reported absence of survivin-2B in some cases of tumor progression, we analyzed the expression of these transcripts in breast cancer.
Material and Methods: reverse transcriptase polymerase chain reaction was performed using RNA samples obtained from 2 groups of breast cancer: node-negative (N−) and locally advanced (LA) tumors.
Results: all survivin variants were expressed in a majority of tumor samples, with survivin variant being the most dominant. In contrast, survivin-2B expression frequency was higher in N− (37/40: 93%) than in LA tumors (56/84: 67%; p=0.002).
Conclusions: These results demonstrated the expression of survivin splice variants in breast tumors and strongly suggested that the absence of expression of survivin-2B could be related to tumor progression in this disease.

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The proteasome inhibitor bortezomib (VELCADE™) sensitizes human tumor cells to TRAIL-mediated apoptosis by reduction of c-FLIP

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We have previously reported that the proteasome inhibitor bortezomib (PS-341, VELCADE™) can sensitize a murine acute myeloid leukemia (C1498) and a murine renal cancer (Renca) to TRAIL-mediated apoptosis. The effects of bortezomid and TRAIL were selective, since this combination could purge C1498 tumor cells from bone marrow, without major effects on normal bone marrow cells. Surprisingly, sensitization of these murine tumor cells was independent of any effect of bortezomib on NF-kB activation, yet did correlate with a reduction in levels of the anti-apoptotic protein c-FLIP. Utilizing the NCI panel of 60 human tumor cell lines, we assessed the sensitivity of a wide variety of different human cancer cell lines to the combination of bortezomid and TRAIL. A significant number of the tumor cell lines (20–30%) were dramatically sensitized to TRAIL-mediated apoptosis by treatment with bortezomib (20nM). However, for the remainder of the tumor cell lines, no such sensitization occurred. No