SCRC - Sheba Cancer Research center
2004 Publications

Articles published during 2004 on the subject of CANCER

Abramovitch R, Itzik A, Harel H, Nagler A, Vlodavsky I, Siegal T.
Halofuginone inhibits angiogenesis and growth in implanted metastatic rat brain tumor model--an MRI study.
Tumor growth and metastasis depend on angiogenesis; therefore, efforts are made to develop specific angiogenic inhibitors. Halofuginone (HF) is a potent inhibitor of collagen type alpha1(I). In solid tumor models, HF has a potent antitumor and antiangiogenic effect in vivo, but its effect on brain tumors has not yet been evaluated. By employing magnetic resonance imaging (MRI), we monitored the effect of HF on tumor progression and vascularization by utilizing an implanted malignant fibrous histiocytoma metastatic rat brain tumor model. Here we demonstrate that treatment with HF effectively and dose-dependently reduced tumor growth and angiogenesis. On day 13, HF-treated tumors were fivefold smaller than control ($P < .001$). Treatment with HF significantly prolonged survival of treated animals (142%; $P = .001$). In HF-treated rats, tumor vascularization was inhibited by 30% on day 13 and by 37% on day 19 ($P < .05$). Additionally, HF treatment inhibited vessel maturation ($P = .03$). Finally, in HF-treated rats, we noticed the appearance of a few clusters of satellite tumors, which were distinct from the primary tumor and usually contained vessel cores. This phenomenon was relatively moderate when compared to previous reports of other antiangiogenic agents used to treat brain tumors. We therefore conclude that HF is effective for treatment of metastatic brain tumors.
Neoplasia. 2004 Sep-Oct;6(5):480-9

Abramovitch R, Tavor E, Jacob-Hirsch J, Zeira E, Amariglio N, Pappo O, Rechavi G, Galun E, Honigman A.
A pivotal role of cyclic AMP-responsive element binding protein in tumor progression.
Tumor microenvironment controls the selection of malignant cells capable of surviving in stressful and hypoxic conditions. The transcription factor, cyclic AMP-responsive element binding (CREB) protein, activated by multiple extracellular signals, modulates cellular response by regulating the expression of a multitude of genes. Previously, we have demonstrated that two cystein residues, at the DNA binding domain of CREB, mediate activation of CREB-dependent gene expression at normoxia and hypoxia. The construction of a dominant-positive CREB mutant, insensitive to hypoxia cue (substitution of two cystein residues at position 300 and 310 with serine in the DNA binding domain) and of a dominant negative CREB mutant (addition of a mutation in serine(133)), enabled a direct assessment, in vitro and in vivo, of the role of CREB in tumor progression. In this work, we demonstrate both in vitro and in vivo that CREB controls hepatocellular carcinoma growth, supports angiogenesis, and renders resistance to apoptosis. Along with the identification, by DNA microarray, of the CREB-regulated genes in normoxia and hypoxia, this work demonstrates for the first time that in parallel to other hypoxia

responsive mechanisms, CREB plays an important role in hepatocellular carcinoma tumor progression.
Cancer Res. 2004 Feb 15;64(4):1338-46

Ashur-Fabian O, Avivi A, Trakhtenbrot L, Adamsky K, Cohen M, Kajakaro G, Joel A, Amariglio N, Nevo E, Rechavi G.
Evolution of p53 in hypoxia-stressed Spalax mimics human tumor mutation.
The tumor suppressor gene p53 controls cellular response to a variety of stress conditions, including DNA damage and hypoxia, leading to growth arrest and/or apoptosis. Inactivation of p53, found in 40-50% of human cancers, confers selective advantage under hypoxic microenvironment during tumor progression. The mole rat, Spalax, spends its entire life cycle underground at decidedly lower oxygen tensions than any other mammal studied. Because a wide range of respiratory adaptations to hypoxic stress evolved in Spalax, we speculated that it might also have developed hypoxia adaptation mechanisms analogous to the genetic/epigenetic alterations acquired during tumor progression. Comparing Spalax with human and mouse p53 revealed an arginine (R) to lysine (K) substitution in Spalax (Arg-174 in human) in the DNA-binding domain, identical to known tumor associated mutations. Multiple p53 sequence alignments with 41 additional species confirmed that Arg-174 is highly conserved. Reporter assays uncovered that Spalax p53 protein is unable to induce apoptosis-regulating target genes, resulting in no expression of apaf1 and partial expression of puma, pten, and noxa. However, cell cycle arrest and p53 stabilization/homeostasis genes were overactivated by Spalax p53. Lys-174 was found critical for apaf1 expression inactivation. A DNA-free p53 structure model predicts that Arg-174 is important for dimerization, whereas Spalax Lys-174 prevents such interactions. Similar neighboring mutations found in human tumors favor growth arrest rather than apoptosis. We hypothesize that, in an analogy with human tumor progression, Spalax underwent remarkable adaptive p53 evolution during 40 million years of underground hypoxic life.
Proc Natl Acad Sci U S A. 2004 Aug 17;101(33):12236-41

Bakhanashvili M, Novitsky E, Lilling G, Rahav G.
P53 in cytoplasm may enhance the accuracy of DNA synthesis by human immunodeficiency virus type 1 reverse transcriptase.
The tumor suppressor protein p53 displays 3' --> 5' exonuclease activity and can provide a proofreading function for DNA polymerases. Reverse transcriptase (RT) of human immunodeficiency virus (HIV)-1 is responsible for the conversion of the viral genomic ssRNA into the proviral DNA in the cytoplasm. The relatively low fidelity of HIV-1 RT was implicated as a dominant factor contributing to the genetic variability of the virus. The lack of intrinsic 3' --> 5' exonuclease activity, the formation of 3'-mispaired DNA and the subsequent extension of this DNA were shown to be determinants for the low fidelity of HIV-1 RT. It was of interest to analyse whether the cytoplasmic proteins may affect the accuracy of DNA synthesis by RT. We investigated the fidelity of DNA synthesis by HIV-1 RT with and without exonucleolytic proofreading provided by cytoplasmic fraction of LCC2 cells expressing high level of wild-type functional p53. Two basic features related to fidelity of DNA synthesis were studied: the misinsertion and mispair extension. The misincorporation of noncomplementary deoxynucleotides into nascent DNA and subsequent mispair extension by HIV-1 RT were substantially decreased in the

presence of cytoplasmic fraction of LCC2 cells with both RNA/DNA and DNA/DNA template-primers with the same target sequence. The mispair extension frequencies obtained with the HIV-1 RT in the presence of cytoplasmic fraction of LCC2 cells were significantly lower (about 2.8-15-fold) than those detected with the purified enzyme. In addition, the productive interaction between polymerization (by HIV-1 RT) and exonuclease (by p53 in cytoplasm) activities was observed; p53 preferentially hydrolyses mispaired 3'-termini, permitting subsequent extension of the correctly paired 3'-terminus by HIV-1 RT. The data suggest that p53 in cytoplasm may affect the accuracy of DNA replication and the mutation spectra of HIV-1 RT by acting as an external proofreader. Furthermore, the decrease in error-prone DNA synthesis with RT in the presence of external exonuclease, provided by cytoplasmic p53, may partially account for lower mutation rate of HIV-1 observed in vivo.
Oncogene. 2004 Sep 9;23(41):6890-9

Bar J, Cohen-Noyman E, Geiger B, Oren M.
Attenuation of the p53 response to DNA damage by high cell density.
The p53 tumor suppressor is critical for preventing cancer progression. Numerous observations suggest that p53 function can be modulated by the cells' microenvironment. We addressed specifically the impact of cell crowding on the induction of p53 by DNA damage. We report that cell crowding attenuates markedly p53 upregulation, transcriptional activation and subsequent p53-dependent apoptosis following exposure to genotoxic stress. The p53 protein remains short-lived in confluent cultures regardless of the extent of DNA damage, even though it undergoes efficient phosphorylation on the mouse equivalent of human p53 serine 15. This inhibitory effect of cell crowding is not a secondary consequence of density-dependent cell cycle arrest (contact inhibition). Microscopic examination indicates that dense cultures display prominent cadherin-mediated cell-cell junctions, and only poor cell-matrix focal adhesions, whereas sparse cells possess conspicuous matrix adhesions and essentially no cell-cell contacts. High-density cell culture might recapitulate the microenvironment of cells in a living organism, where the response of p53 to DNA damage is reported to be low in some organs and ages. The impact of cell density on p53 activation may have important bearings on the involvement of p53 in tumor suppression and the cellular response to anticancer therapy.
Oncogene. 2004 Mar 18;23(12):2128-37

Barda G, Menczer J, Chetrit A, Lubin F, Beck D, Piura B, Glezerman M, Modan B, Sadetzki S.
National Israel Ovarian Cancer Group.omparison between primary peritoneal and epithelial ovarian carcinoma: a population-based study.
OBJECTIVE: This study was undertaken to characterize primary peritoneal carcinoma (PPC) compared with ovarian carcinoma (OvC). STUDY DESIGN: Within the framework of a nationwide epidemiologic Israeli study, 95 PPC patients were identified and compared with 117 FIGO stage III-IV epithelial OvC patients matched by age and continent of birth. Data were abstracted from medical records and personal interviews. RESULTS: Our data confirm the similarities between PPC and OvC. A higher rate of abdominal distention, volume of ascites, and malignant cells in ascitic fluid and lower rate of pelvic palpable mass and personal breast cancer history were found in the PPC compared with the OvC group. The overall survival was similar in

both groups (30-33 months). In optimally cytoreduced patients, survival was better in the OvC group. Diameter of residual disease was associated with better survival only in the OvC group. CONCLUSION: The clinical differences do not enable a preoperative distinction between the neoplasms.
Am J Obstet Gynecol. 2004 Apr;190(4):1039-45

Beiner ME, Gotlieb WH, Korach Y, Shrim A, Stockheim D, Segal Y, Fridman E, Ben-Baruch G.
Cystectomy for immature teratoma of the ovary.
OBJECTIVES: Most patients with malignant ovarian germ cell tumors (MOGCT) of the ovary are in their reproductive years and wish to preserve fertility. Because of the excellent response to chemotherapy, the standard of care is unilateral salpingo-oophorectomy (USO), but some patients undergo cystectomy only before final pathology. In view of the lack of information concerning the outcome following cystectomy in germ cell tumors, we retrospectively evaluated the clinical outcome of patients who underwent cystectomy only as part of their surgical treatment. METHODS: The clinical and pathological records of 38 patients diagnosed with MOGCT, treated and followed in the department of gynecologic oncology were reviewed. Eight patients underwent cystectomy only at their initial surgery and are the subjects of this study. RESULTS: All the eight patients who underwent cystectomy were diagnosed with immature teratoma (three grade 1, four grade 2, and one grade 3) on final pathology following surgery. All except three patients (two with grade 1 and one with grade 2 disease) received adjuvant chemotherapy. Follow-up was available for all the patients, with a median duration of 4.7 years. No recurrences were observed during this period. Three patients delivered a total of seven babies. CONCLUSIONS: Cystectomy followed by adjuvant chemotherapy appeared satisfactory for apparent early-stage immature teratoma when close follow-up was carried out. It is still unclear whether cystectomy alone will also be safe.
Gynecol Oncol. 2004 May;93(2):381-4

Bercovitch M, Adunsky A.
Patterns of high-dose morphine use in a home-care hospice service: should we be afraid of it?
BACKGROUND: Management of cancer pain is one of the most important goals of palliative care. Relieving pain is often problematic. High doses of morphine at home may be required to relieve patients' pain, and is therefore feared. The goals of the current study were to assess the feasibility of high-dose morphine use at home, to characterize the patients, and to examine whether the use of high-dose morphine might affect their survival. METHODS: The authors retrospectively studied the medical charts of 661 outpatients, which were completed by a home-care hospice team. The authors collected data regarding demographic parameters, medical diagnosis, pain type, morphine dosage, use of rescue doses in addition to regular doses, use of coanalgesics and adjuvant treatments, and survival time as associated with morphine dosage. The authors also compared the data of patients receiving high-dose morphine with those of a group of patients receiving regular doses. RESULTS: The authors identified 435 patients (65.8%) who received morphine for pain relief. Of these, 396 patients (91%) received a dose of 5-299 mg of morphine per day), 32 patients (7.4%) received 300-599 mg of morphine per day), and 7 patients (1.6%) received very high doses (> or = 600 mg of morphine per day). Overall, 39 patients (9%) received > 299 mg per day. Morphine dosage was found to be inversely

correlated (r) with age (r = -0.254; P < 0.001). Male patients required slightly higher dosages than female patients (62.5% of high-dose and 71% of very high-morphine groups, respectively). Primary gastrointestinal (P = 0.015) and lung (P = 0.027) carcinomas, as well as metastatic bone disease (P = 0.001), ovarian carcinoma (P = 0.037), and brain tumors (P = 0.0053) were associated with higher and very higher morphine dosages. Adverse effects were similar in the groups receiving regular, high, and very high doses of morphine. The median survival of patients treated with high doses of morphine was 27 days and was 37 days for those treated with very high doses. Patients treated with low doses of morphine survived for 18 days. Patients not treated with morphine survived for 22 days (P = 0.001 by Mantel-Cox analysis; P = 0.029 by Breslow analysis). CONCLUSIONS: The use of high and very high morphine doses at home proved safe and did not appear to affect the patients' life expectancy adversely. The use of high or very high-dose morphine should not be a barrier to providing palliative terminal care for home-care hospice patients.
Cancer. 2004 Sep 15;101(6):1473-7

Bielorai B, Hughes MR, Auerbach AD, Nagler A, Loewenthal R, Rechavi G, Toren A.

Successful umbilical cord blood transplantation for Fanconi anemia using preimplantation genetic diagnosis for HLA-matched donor.

Fanconi anemia is a rare autosomal recessive disease characterized by bone marrow failure, developmental anomalies, and a high incidence of myelodysplasia and acute myeloid leukemia. Stem cell transplantation is the only curative treatment. In the absence of matched- sibling donor, an alternative mismatched family or matched unrelated donor can be used, but the results are inferior to the matched-sibling transplant and carry a high risk of morbidity and mortality. Preimplantation genetic diagnosis (PGD) has been increasingly used in recent years for mutation analysis for many genetic disorders and results in the birth of healthy children, saving the need for the termination of pregnancy of an affected embryo. The use of PGD for combined analysis of mutation and HLA-matching was reported for the first time in 2001. This enables the birth of an unaffected child who can serve as a donor for an affected sibling in need for stem cell transplantation. We report successful cord blood transplantation for a Fanconi anemia patient from his HLA-matched sibling, born after PGD that included mutation analysis for Fanconi anemia and HLA typing. PGD can provide an unaffected donor for a sibling affected by genetic disease in the absence of a compatible related donor.
Am J Hematol. 2004 Dec;77(4):397-9

Bielorai B, Trakhtenbrot L, Amariglio N, Rothman R, Tabori U, Dallal I, Golan H, Neumann Y, Reichart M, Kaplinsky C, Rechavi G, Toren A.

Multilineage hematopoietic engraftment after allogeneic peripheral blood stem cell transplantation without conditioning in SCID patients.

Successful stem cell transplantation for patients with severe combined immunodeficiency (SCID) from matched family donors without conditioning results in engraftment of T lymphocytes. B lymphocytes engraft in only 50% of the cases, while myelopoiesis and erythropoiesis remain of host origin. Full hematopoietic engraftment was reported in one case after bone marrow transplantation without conditioning for a SCID patient. We studied three SCID patients who were transplanted with unmodified mobilized peripheral blood from HLA-identical family sex-mismatched members. They received megadoses of stem cells (18-23 x

10(6)CD34/kg). In contrast to the expected mixed chimerism that usually occurs in the absence of conditioning, we found in our patients 100% donor cell engraftment based on fluorescence in situ hybridization (FISH) and microsatellite techniques. Subset analysis of the engrafted cells using a multiparametric system enabling a combined analysis of morphology, immunophenotyping and FISH showed that both T and B lymphocytes and myeloid cells were of donor origin in two patients, while T lymphocytes and myeloid cells were of donor origin in the third. In the two cases with ABO incompatibility, erythroid engraftment was evidenced by blood group conversion from recipient to donor type. Multilineage donor engraftment is possible in SCID patients even without conditioning.
Bone Marrow Transplant. 2004 Aug;34(4):317-20

Blank M, Lavie G, Mandel M, Hazan S, Orenstein A, Meruelo D, Keisari Y.
Antimetastatic activity of the photodynamic agent hypericin in the dark.
A unique property of the photodynamic signal transduction inhibitor hypericin (HY) is high functionality in the dark, which has been shown to result in portfolio of anticancer activities both in vitro and in vivo. Here we show that treatment with HY significantly reduces growth rate of metastases in 2 murine models: breast adenocarcinoma (DA3) and squamous cell carcinoma (SQ2). Focus on metastases was achieved by resection of primary tumors at stages in which micrometastases exist in lungs. Long-term animal survival in DA3 tumor-excised groups increased from 15.6% in controls to 34.5% following supplementary treatment with HY. In mice bearing SQ2 tumor metastases, therapy with HY increased animal survival from 17.7% in controls to 46.1%. Using Laser-induced fluorescence and multipixel spectral image analyses, we demonstrate that HY has a high tendency to accumulate in primary and metastatic tumors; HY content in lungs bearing metastases was approximately 2-fold higher than in the lungs of healthy animals. The tendency of HY to preferentially concentrate in lung metastases, combined with its potent antiproliferative activities, may render HY as a useful supplementary modality in the treatment of metastatic cancer irrespective of photoactivation.
Int J Cancer. 2004 Sep 10;111(4):596-603

Braun M, Hasson-Ohayon I, Perry S, Kaufman B, Uziely B.
Motivation for giving birth after breast cancer.
Background: Breast cancer at a young age threatens the natural developmental tasks that characterize this phase in life including parenthood. The dilemma of whether to give birth arises due to the potential medical, psychological and social implications of pregnancy, birth and child rearing after breast cancer. The purpose of this study was to investigate the positive and negative motivations toward childbirth of breast cancer survivors and their husbands.Method: Thirty breast cancer survivors and 13 husbands were compared to 29 healthy women and 15 husbands. The study included qualitative questions and quantitative measures including: a demographic and medical questionnaire, the Parenthood Motivation Questionnaire-Revised, the ENRICH Marital Satisfaction Scale, the Brief Symptom Inventory, the Impact of Events Scale, and the Mental Adjustment to Cancer Questionnaire.Results: The experience of having breast cancer did not hinder overall positive motivations toward childbirth, nor did it increase overall negative motivations toward childbirth, among women and their husbands. However, there were several differences between the groups, which may reflect the illness experience. For example, breast cancer survivors and their husbands

reported more negative motivations toward childbirth due to health concerns than did healthy women and their husbands.
Psychooncology. 2004 Sep 13; [Epub ahead of print]

Bruchim I, Fishman A, Friedman E, Goldberg I, Chetrit A, Barshack I, Dekel E, Hirsh-Yechezkel G, Modan B, Kopolovic J.
Analyses of p53 expression pattern and BRCA mutations in patients with double primary breast and ovarian cancer.
OBJECTIVE: To analyze the somatic pattern of p53 expression and BRCA germline mutation status in Israeli patients with both ovarian (OvCa) and breast cancer (BrCa). METHODS: The study group comprised 43 Israeli patients with OvCa, all of whom had previous primary BrCa. p53 immunohistochemistry (IHC) on all available archival tissues and genotyping for the three predominant Jewish germline BRCA1-2 mutations were carried out. Samples from 64 patients with solitary OvCa and 61 with solitary BrCa were similarly analyzed as controls. RESULTS: p53 expression pattern and the immunopositivity rate were similar in the ovarian and breast tumors within the study group and in the two control groups: positive p53 staining was detected in 68% of ovarian tumors in the study group compared with 71.9% in the controls, and in 19.4% of the BrCa tissues versus 21.3% in the controls. Within the study group, advanced stage OvCa had a higher rate of p53 expression (84%) compared to early stage disease (38.5%) (P = 0.006). This difference was not apparent in the solitary OvCa control group. OvCa in BRCA1-2 mutation carriers from the study group were more likely to display positive p53 staining (79%), especially in tumors diagnosed before the age of 60 (90%) compared with the OvCa of noncarriers (60%), but this difference was statistically insignificant. The p53 expression rate in BrCa samples from the study group was not associated with BRCA1-2 mutation status. CONCLUSIONS: Positive p53 expression, detected by IHC, in OvCa patients with previous primary BrCa is significantly higher in advanced stage disease in BRCA1-2 mutation carriers. There is a higher positive p53 expression somatically in OvCa in BRCA1-2 carriers in whom OvCa was diagnosed before the age of 60 years, although this trend is not statistically significant. These observations suggest that somatic p53 inactivation may be an important event in ovarian tumorigenesis in this subset of patients.
Int J Gynecol Cancer. 2004 Mar-Apr;14(2):251-8

Cohen N, Betts DR, Tavori U, Toren A, Ram T, Constantini S, Grotzer MA, Amariglio N, Rechavi G, Trakhtenbrot L.
Karyotypic evolution pathways in medulloblastoma/primitive neuroectodermal tumor determined with a combination of spectral karyotyping, G-banding, and fluorescence in situ hybridization.
Medulloblastomas (MBs) or primitive neuroectodermal tumors (PNETs) represent 15%-30% of pediatric brain tumors and are the most common brain tumors in children; they are rare in adults. Classification of these tumors is based on tissue morphology and is often controversial and problematic. Karyotypic analysis of these tumors using conventional cytogenetic methods is often a difficult process that may be hindered by a limited number of metaphase cells and poor chromosome morphology, often leading to only partial characterization of the chromosomal abnormalities. We investigated three primary human tumors and four cell lines (CHO-707, DAOY, D-341, and PFSK) utilizing a combination of conventional G-banding, spectral karyotyping (SKY), and fluorescence in situ hybridization (FISH) techniques.

A high level of intratumoral heterogeneity was seen, with multiple numerical and structural chromosomal aberrations. The chromosomes most frequently involved in structural aberrations were chromosomes 1 (14 rearrangements), 7 (9 rearrangements), and 21 (9 rearrangements). The chromosomes most frequently involved in numerical aberrations were chromosomes 1, 12, and 13 (four cases) and chromosomes 14, 17, 19, 21, 22, and X (three cases). Numerous aberrant chromosomes were characterized only with the SKY analysis, and based on these findings multiple clones were identified, facilitating analysis of karyotypic evolution. The most frequent evolution mechanism was via polyploidization, followed by acquisition of additional numerical or structural aberrations (or both); however, the results showed that the karyotypic evolution process in these tumors is typically divergent and complex.
Cancer Genet Cytogenet. 2004 Feb;149(1):44-52

Cohen O, Dabhi S, Karasik A, Zila Zwas S.
Compliance with follow-up and the informative value of diagnostic whole-body scan in patients with differentiated thyroid carcinoma given recombinant human TSH.
OBJECTIVE: Protocols for monitoring patients with differentiated thyroid cancer (DTC) include measurement of serum Tg and, for most patients, whole-body scan (WBS) with low radioiodine activities ('diagnostic' WBS). Recently, recombinant human thyroid-stimulating hormone (rhTSH) has become available to provide the TSH stimulation necessary for these procedures, whilst avoiding thyroid hormone withdrawal and hypothyroid complications. In addition, the inclusion of diagnostic WBS in DTC follow-up has recently become controversial. We have assessed the compliance with withdrawal-aided monitoring and the informative value of diagnostic WBS in consecutive tertiary referral center patients. DESIGN: Forty-eight patients received rhTSH (0.9 mg) in two consecutive daily injections, with radioiodine administration 24 h, diagnostic WBS 48 h, and serum Tg testing prior to and 72 h later. METHODS: Compliance with withdrawal-aided monitoring was assessed with a questionnaire provided by the referring physician, patient record analysis, and patient interview. The informative value of diagnostic WBS was assessed by comparing findings against serum Tg measurements in light of physical and other radiological examinations. RESULTS: Forty of the forty-eight patients were female, the mean age was 43.9 years and the median follow-up from diagnosis was 4.5 years (range 1-19 years). Twenty-seven (56%) patients were compliant and 12 (25%) were non-compliant; compliance was not known in nine. Of 17 patients with clinically suspicious or significant findings on any available modality, four had uptake outside the thyroid bed on WBS but stimulated Tg <2.5 ng/ml on immunometric assay, while five had a negative WBS with serum Tg >2.5 ng/ml. CONCLUSIONS: Thyroid hormone withdrawal substantially impairs, and rhTSH administration substantially promotes, compliance with DTC monitoring. rhTSH-aided WBS is informative and should be included in the follow-up of unselected patients with DTC.
Eur J Endocrinol. 2004 Mar;150(3):285-90.

Cohen Y, Amir G, Schibi G, Amariglio N, Polliack A.
Rapidly progressive diffuse large B-cell lymphoma with initial clinical presentation mimicking seronegative Wegener's granulomatosis.
Here we present a 40-yr-old male patient with an aggressive B-cell lymphoma, who presented 2 yr earlier with polyarthritis, and was responsive to steroids and oral

methotrexate. Thereafter he developed skin and lung lesions which on biopsy consisted of mixed 'inflammatory' infiltrates with granulomatous vasculitis. A diagnosis of seronegative Wegener's granulomatosis was made and the patient received a combination of prednisone and cyclophosphamide with clinical improvement and clearance of the radiological lesions in the lungs. The patient was now completely asymptomatic for 1 yr, but then generalized lymphadenopathy appeared, which was shown by histopathology to be large B-cell lymphoma, also involving the bone marrow. Despite intensive chemotherapy, his disease could not be controlled because of primary chemoresistance, which was perhaps in some way related to exposure to the suboptimal doses of chemotherapy given during the 'inflammatory' period before the diagnosis of lymphoma was established. This case illustrates the occasional difficulty in distinguishing between extranodal lymphoproliferative diseases and autoimmune disorders especially when clonality cannot be proved. It also shows the possible risk of 'masking' a true lymphoma by treating non-malignant diseases with immunosuppressive agents, which may eventually contribute to the development of chemoresistant lymphoma.
Eur J Haematol. 2004 Aug;73(2):134-8

Cohen Y, Nagler A.
Umbilical cord blood transplantation--how, when and for whom?
In recent years, umbilical cord blood (UCB) has emerged as a feasible alternative source of hematopoietic progenitors (CD34+) for allogeneic stem cell transplantation, mainly in patients who lack HLA-matched marrow donors. Since the first case reported in 1998, more than 3500 patients have received UCB transplants for a variety of malignant and non-malignant diseases. The vast majority of recipients were children with an average weight of 20 kg; however, more than 500 UCB transplantations (UCBTs) have already been performed in adults. The "naive" nature of UCB lymphocytes also permits the use of HLA-mismatched grafts at 1-2 loci without higher risk for severe graft versus host disease (GvHD) relative to bone marrow transplantation (BMT) from a full matched unrelated donor. Furthermore, UCB is rich in primitive CD16(-)CD56++ NK cells, which possess impressive proliferative and cytotoxic capacities and can be induced to expand using IL-12 or IL-15, so as to mount a substantial graft versus leukemia (GvL) effect. The main disadvantage of UCB is the low stem cell yields, resulting in higher rates of graft failure as well as delayed time to engraftment compared to BMT. One rational approach to overcome this limitation involves ex vivo expansion of UCB derived hematopoietic precursors. In this review we tried to answer the question: UCBT how, when and for whom. This procedure is mostly applicable for children and especially those with indication for full allogeneic transplantation but who lack a matched sibling donor. Experimental approaches including ex vivo expansion of CB with cocktail of hematopoietic growth factors, with or without differentiation blocking agents, co-transplantation of haploidentical and CB cells or co-transfusion of CB and mesenchymal cells may enable successful UCBT in adults and probably will result in expanding the indication to solid tumors or autoimmune disorders.
Blood Rev. 2004 Sep;18(3):167-79.

Cohena Y, Nagler A.
Hematopoietic stem-cell transplantation using umbilical-cord blood.
In the recent years, umbilical cord blood (UCB) has emerged as an alternative source of hematopoietic progenitors (CD34+) for allogeneic stem cell transplantation, mainly

in patients lacking an HLA-matched marrow donor. Since 1998, about 2500 patients have received UCB transplants for a variety of malignant and non-malignant diseases. The vast majority of recipients were children with an average weight of 20kg, however, more than 500 UCB transplantations (UCBT) have already been performed in adults. The "naive" nature of UCB lymphocytes may explain the lower incidence and severity of graft vs. host disease (GvHD) encountered in UCBT compared to the allogeneic transplant setting. Furthermore, UCB is rich in primitive CD16-CD56++ NK cells, which possess significant proliferative and cytotoxic capacities and can be expanded using IL-12 or IL-15, so as to mount a substantial graft vs. leukemia (GvL) effect. The major disadvantage of UCB is the low yield of stem cells, resulting in higher rates of engraftment failure and slower time to engraftment compared to bone marrow transplantation (BMT). A rational approach thus involves ex vivo expansion of UCB derived hematopoietic precursors.
Leuk Lymphoma. 2003 Aug;44(8):1287-99

Czyz J, Dziadziuszko R, Knopinska-Postuszuy W, Hellmann A, Kachel L, Holowiecki J, Gozdzik J, Hansz J, Avigdor A, Nagler A, Osowiecki M, Walewski J, Mensah P, Jurczak W, Skotnicki A, Sedzimirska M, Lange A, Sawicki W, Sulek K, Wach M, Dmoszynska A, Kus A, Robak T, Warzocha K.
Outcome and prognostic factors in advanced Hodgkin's disease treated with high-dose chemotherapy and autologous stem cell transplantation: a study of 341 patients.
BACKGROUND: The reported probability of survival of patients with Hodgkin's disease (HD) following high-dose chemotherapy with autologous stem cell transplantation (HDC/ASCT) is 35-65% at 5 years. The Polish Lymphoma Research Group investigated retrospectively prognostic factors for overall survival (OS) and event-free survival (EFS), and the risk of secondary malignancies in a large series of patients who underwent HDC/ASCT. PATIENTS AND METHODS: The data of 341 consecutive patients treated in 10 centers from 1990 to 2002 were collected and analyzed. RESULTS: The actuarial 5-year OS and EFS were 64% [95% confidence interval (CI) 57% to 71%] and 45% (95% CI 39% to 51%), respectively. In the multivariate model, unfavorable prognostic factors for EFS were less than partial response at the time of ASCT [relative risk (RR), 2.92 (95% CI 1.68-5.08); P<0.001] and three or more previous chemotherapy lines (RR, 2.16; 95% CI 1.42-3.30; P<0.001). These two factors were also associated with unfavorable OS (RR, 3.32; 95% CI 1.90-5.79; P<0.001 and RR, 2.34, 95% CI 1.51-3.64; P<0.001). Five-year cumulative risk of secondary malignancy was 8.4% (95% CI 2% to 13%) and the only identified risk factor was splenectomy (P=0.02). CONCLUSIONS: HDC/ASCT should be considered early in the course of disease for patients with a response after standard therapy.
Ann Oncol. 2004 Aug;15(8):1222-30

Czyz J, Szydlo R, Knopinska-Posluszny W, Hellmann A, Gozdzik J, Hansz J, Smolewski P, Robak T, Osowiecki M, Walewski J, Avigdor A, Nagler A, Zemelka T, Pawlicki M, Sawicki Z, Wojtukiewicz M, Kachel L, Holowiecki J, Charlinski G, Jedrzejczak WW.
Treatment for primary refractory Hodgkin's disease: a comparison of high-dose chemotherapy followed by ASCT with conventional therapy.
Our previously published study showed promising results of autologous stem cell transplantation (ASCT) in patients with primary resistant Hodgkin's disease (HD).

Probabilities of overall survival (OS) and progression-free survival (PFS) at 3 years were 55 and 36%, respectively. The present study was undertaken to compare these results with conventionally treated patients and thus evaluate therapeutic options. Retrospective data on 76 adult patients who underwent ASCT were matched with 76 conventionally treated patients from 17 centers. Comparison of clinical characteristics in both groups showed that ASCT patients were younger (24 vs 31.5 years, P=0.001), more frequently presented with 'B' symptoms (P=0.03) and that more patients treated with chemotherapy (CT) had elevated LDH (P=0.03). In univariate analyses, bulky disease (P=0.0043) and complete resistance to standard CT (P=0.051) were found to be risk factors for OS. In a multivariate survival analysis only bulky disease was found to an independent prognostic factor (P=0.005). There was no difference in survival between the treatment groups with 5 years OS 33.7 (CI: 23-46) in the ASCT group and 35.6% (CI: 25-50) for the CT group (P=0.92). We conclude that ASCT is not superior to standard CT for treatment of patients with primary refractory HD.
Bone Marrow Transplant. 2004 Jun;33(12):1225-9

Davidson B, Goldberg I, Tell L, Vigdorchik S, Baekelandt M, Berner A, Kristensen GB, Reich R, Kopolovic J.

The clinical role of the PEA3 transcription factor in ovarian and breast carcinoma in effusions.

Ets transcription factors play a central role in invasion and metastasis through regulation of synthesis of proteolytic enzymes and angiogenic molecules. The objective of this study was to investigate the role of PEA3 in tumor progression of ovarian and breast carcinoma metastatic to effusions, and to evaluate the expression of Ets-2 and Erg in ovarian carcinoma. Ovarian (83 malignant effusions, 102 corresponding solid lesions) and breast (33 malignant effusions, 40 corresponding solid lesions) carcinomas were evaluated for expression of PEA3 using mRNA in situ Hybridization (ISH). Expression of Ets-2 and Erg mRNA was analyzed in 50 ovarian carcinoma effusions using the same method. PEA3 mRNA expression was comparable at all sites in ovarian carcinoma (44 out of 83; 53% of effusions, 48 out of 102; 47% of solid tumors). PEA3 mRNA expression in effusions correlated with mRNA expression of the previously studied alphav (P = 0.022), alpha6 (P < 0.001) and beta1 (P < 0.001) integrin subunits, the matrix metalloproteinase (MMP) inducer EMMPRIN (P = 0.015) and interleukin-8 (IL-8) (P = 0.033). Erg and Ets-2 mRNA was expressed in 15 out of 50 (30%) and 18 out of 50 (36%) effusions, respectively, and co-localized with PEA3 (P = 0.017 for Erg, P = 0.004 for Ets-2). In breast carcinoma, PEA3 expression was seen in 19/40 (48%) of solid lesions, with a significant upregulation in corresponding effusions compared to primary tumors (24 out of 33; 73%, P = 0.038). PEA3 mRNA expression in effusions obtained prior to the institution of chemotherapy predicted significantly shorter overall survival in univariate analysis (24 vs 37 months, P = 0.03), with a similar trend for Erg (13 vs 30 months, P = 0.1). In conclusion, PEA3 is expressed at all anatomic sites in serous ovarian cancer and co-localizes with Erg, Ets-2 and several metastasis-associated molecules. PEA3 mRNA expression is a novel marker for tumor progression to malignant effusion in breast carcinoma, and predicts poor outcome in effusions sampled prior to therapeutic intervention in ovarian carcinoma. These findings support a biological role for Ets transcription factors in these malignancies and suggests that they may be targets for therapeutic intervention.
Clin Exp Metastasis. 2004;21(3):191-9.

Dekel B, Hochman E, Sanchez MJ, Maharshak N, Amariglio N, Green AR, Izraeli S.

Kidney, blood, and endothelium: developmental expression of stem cell leukemia during nephrogenesis.

BACKGROUND: In vertebrates the hematopoietic and renal tissues share a common mesodermal origin. Recently, we have analyzed global gene expression during human nephrogenesis and observed up-regulation of stem cell leukemia (SCL), a transcription factor critical for hematopoietic and endothelial lineage specification. Here we characterize the expression of SCL along with its distinct 3' hematopoietic and endothelial enhancer (SCL 3'En) during kidney development. METHODS: mRNA and protein expression of SCL were examined in developing murine and human kidneys by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry. The activity of SCL 3'En was examined by X-galactosidase (X-gal) staining of embryonic kidneys obtained from SCL +6E5/lacZ/3'En transgenic mice and by reporter lacZ assay in various renal cell lines. RESULTS: We found developmental regulation of SCL mRNA with highest levels of expression in embryonic day 17 (E17) mouse kidneys and lowest in postnatal and adult kidneys. Immunostaining of human fetal kidneys demonstrated the protein predominantly in the nephrogenic cortex and particularly in mesenchymal cells and developing glomeruli. Similarly, SCL +6E5/lacZ/3'En transgenic kidneys showed prominent lacZ staining in cells resembling undifferentiated mesoderm cells in close proximity to S and comma-shaped primitive nephrons and in peritubular and glomerular vessel endothelium. The SCL 3'En was activated in the human embryonic kidney cell line (HEK 293), but not in cell lines derived from adult kidney. CONCLUSION: These observations suggest a possible role for SCL in renal vasculogenesis. Undifferentiated mesenchymal cells expressing SCL during early nephrogenesis might represent putative progenitors that can simultaneously give rise to kidney, blood, and endothelium.

Kidney Int. 2004 Apr;65(4):1162-9.

Dekel B, Reisner Y.

Embryonic committed stem cells as a solution to kidney donor shortage.

The number of human kidney transplants has increased rapidly in recent years, but the need greatly exceeds organ availability. Induction of appropriate kidney differentiation and growth from stem or progenitor cell populations represents an attractive option to combat chronic kidney donor shortage. In an analogy to haematopoietic stem cells, which are much more efficient in giving rise to blood than to other cell types, if any at all, renal stem cells could afford an unlimited source for regenerating nephrons. While a single nephrogenic stem cell has not been characterised, indirect evidence suggests that a renal stem cell population is contained within the metanephric mesenchyme, which along with a branch of the Wolffian duct represents the direct precursor of the mature kidney. Human tissue fragments derived from these developing precursors can regenerate renal structures when grafted into mice. Moreover, recent data pinpoints a window of time in human and pig kidney development that may be optimal for transplantation into mature recipients. 'Window' transplants are defined by their remarkable ability to grow, differentiate and undergo vascularisation, achieving successful organogenesis of urine-producing miniature kidneys with no evidence of transdifferentiation into non-renal cell types, lack of tumourigenicity and reduced immunogenicity compared with adult counterparts. In contrast, 'non-window' transplants (earlier or later in gestation) can form teratomas or

are more prone to immune rejection and are less suitable for organogenesis. Hopefully, the use of stage-specific early human and porcine kidney precursors to cultivate mature kidney cells in vivo, possibly in conjunction with other modalities of stem cell technology and tissue engineering, will prove valuable to sustain life in patients with failing kidneys.
Expert Opin Biol Ther. 2004 Apr;4(4):443-54.

Dotan ZA, Dotan A, Ramon J, Avivi L.
Altered mode of allelic replication accompanied by aneuploidy in peripheral blood lymphocytes of prostate cancer patients.
Replication timing of the genetic material is a highly programmed process correlated with expression, stability and methylation capacity. An important aspect of that timing is the temporal order of allelic replication: a synchronous mode for biallelically expressed genes and an asynchronous for monoallelically expressed genes. Previous studies showed that malignancy is associated with changes in the inherent mode of allelic replication, and even normal cells of cancer patients display alterations in the replication of various genes. Using fluorescence in situ hybridization (FISH), we checked whether allelic-replication mode differentiates cancer patients from healthy individuals. We focused on prostate cancer (CAP), the most common diagnosed cancer and the second leading cause of cancer death in men over 50 years old. Five nonrelated genes and a nontranscribed DNA sequence associated with chromosomal segregation were used in our study. All 6 tested loci displayed in peripheral blood lymphocytes stimulated with phytohemagglutinin (PHA) of CAP patients loss of their inherent temporal order of allelic replication, coupled with aneuploidy, the outcome of chromosome malsegregation. The replication-timing modification is a reversible epigenetic alteration, evidenced by our ability to resurrect the normal pattern in all 6 tested loci by introducing an inhibitor of methyl transferase. On the other hand, the methylation-blocking agent failed to obliterate aneuploidy. The replication alteration accompanied by aneuploidy, detected in peripheral blood cells, distinguishes between CAP patients and individuals with benign prostate hyperplasia (BPH; a common disorder in elderly men) better than the routinely used blood marker, the prostate-specific antigen (PSA).
Int J Cancer. 2004 Aug 10;111(1):60-6

Elhasid R, Sahar D, Merling A, Zivony Y, Rotem A, Ben-Arush M, Izraeli S, Bercovich D, Larisch S.
Mitochondrial pro-apoptotic ARTS protein is lost in the majority of acute lymphoblastic leukemia patients.
Acquired resistance towards apoptosis is the hallmark of most if not all types of cancer. We have previously identified and characterized ARTS, a broadly expressed protein localized to mitochondria. ARTS was initially shown to mediate TGF-beta induced apoptosis. Recently, we have found that high levels of ARTS induce apoptosis without additional pro-apoptotic stimuli. Further, ARTS promotes apoptosis in response to a wide variety of pro-apoptotic stimuli. Here, we report that the expression of ARTS is lost in all lymphoblasts of more than 70% of childhood acute lymphoblastic leukemia (ALL) patients. The loss of ARTS is specific, as the related non-apoptotic protein H5, bearing 83% identity to ARTS, is unaffected. During remission, ARTS expression is detected again in almost all patients. Two leukemic cell lines, ALL-1 and HL-60 lacking ARTS, were resistant to apoptotic induction by

ara-C. Transfection of ARTS into these cells restored their ability to undergo apoptosis in response to this chemotherapeutic agent. We found that methylation process contributes to the loss of ARTS expression. We conclude that the loss of ARTS may provide a selective advantage for cells to escape apoptosis thereby contributing to their transformation to malignant lymphoblasts. We therefore propose that ARTS can function as a tumor suppressor protein in childhood ALL.
Oncogene. 2004 Jul 15;23(32):5468-75

Engelmann H, Aderka D, Wallach D.
Purification of TNF binding proteins.
The finding that the two tumor necrosis factor receptors (TNFR) exist in soluble form in various body fluids not only has substantiated the paradigm of naturally existing soluble cytokine receptors but also has represented a milestone on the road to the biochemical and biological characterization of the two TNFRs. This chapter gives a simple, basic protocol for the purification of the two soluble TNFRs. The protocols found here may be easily adapted for the purification of various other soluble cytokine receptors. The purified proteins may be used in biological experiments or for the generation of specific research tools such as polyclonal or monoclonal antibodies.
Methods Mol Med. 2004;98:23-32

Enk CD, Shahar I, Amariglio N, Rechavi G, Kaminski N, Hochberg M.
Gene expression profiling of in vivo UVB-irradiated human epidermis.
BACKGROUND: Several recent studies have employed microarray profiling to study UVB-regulated gene expression in human skin. These studies are all based on UV-irradiated cultured cells that differ substantially from the intact tissues they are supposed to imitate. The purpose of the present study was to analyze the differential expression of UVB-regulated genes in intact human epidermis following in vivo UV irradiation. METHODS: The forearms of human volunteers were exposed to 4 MED of UVB in vivo, followed by removal of epidermal samples from exposed and non-exposed areas after 24 h. RNA samples were analyzed using oligonucleotide microarray (Affymetrix) technology analyzing 12 500 genes simultaneously. Verification of selected genes was performed by semi-quantitative reverse transcriptase polymerase chain reaction. RESULTS: Gene expression patterns clearly distinguished UV-exposed epidermis from unexposed skin. Classification of these genes into functional categories revealed that several biological processes are globally affected by UVB. Significant changes were seen in more than 800 genes. CONCLUSION: Human intact epidermis responds to a single low dose of in vivo UVB irradiation by differential regulation of numerous genes. Our results illustrate the power of global gene expression analysis of human epidermis to identify molecular pathways involved in UV-induced photodamage.
Photodermatol Photoimmunol Photomed. 2004 Jun;20(3):129-37.

Erez A, Perelman M, Hewitt SM, Cojacaru G, Goldberg I, Shahar I, Yaron P, Muler I, Campaner S, Amariglio N, Rechavi G, Kirsch IR, Krupsky M, Kaminski N, Izraeli S.
Sil overexpression in lung cancer characterizes tumors with increased mitotic activity.
Sil (SCL interrupting locus) was cloned from the most common chromosomal rearrangement in T-cell acute lymphoblastic leukemia. It is an immediate early gene whose expression is associated with cell proliferation. Sil protein levels are tightly

regulated during the cell cycle, reaching peak levels in mitosis and disappearing on transition to G1. A recent study found Sil to be one of 17 genes whose overexpression in primary adenocarcinomas predicts metastatic spread. We hypothesized that Sil might have a role in carcinogenesis. To address this question, we utilized several approaches. Using a multitumor tissue array, we found that Sil protein expression was increased mostly in lung cancer, but also at lower levels, in a subset of other tumors. Microarray gene expression analysis and immunohistochemistry of lung cancer samples verified these observations. Sil gene expression in lung cancer correlated with the expression of several kinetochore check-point genes and with the histopathologic mitotic index. These observations suggest that overexpression of the Sil gene characterizes tumors with increased mitotic activity.
Oncogene. 2004 Jul 8;23(31):5371-7

Franitza S, Grabovsky V, Wald O, Weiss I, Beider K, Dagan M, Darash-Yahana M, Nagler A, Brocke S, Galun E, Alon R, Peled A.
Differential usage of VLA-4 and CXCR4 by CD3+CD56+ NKT cells and CD56+CD16+ NK cells regulates their interaction with endothelial cells.
The mechanism that regulates the preferential accumulation of NKT cells in the BM is unknown. The BM endothelium constitutively expresses selectins, the integrin ligands VCAM-1 and ICAM-1, and the chemokine CXCL12. Both NK and NKT subsets of cells exhibited similar tethering and rolling interactions on both P-selectin and E-selectin and expressed similar levels of the integrins, VLA-4 and LFA-1. Although NKT cells express higher levels of CXCR4 than NK cells, CXCL12 (the ligand for CXCR4) rapidly stimulates similar levels of adhesion of NK and NKT cells to VCAM-1 and ICAM-1. In both subsets, the arrest on VCAM-1 was dependent on high affinity VLA-4 and the homing of these cells to the BM of NOD/SCID was VLA-4-dependent. However, as opposed to the situation for NK cells, CXCL12 preferentially triggers, under shear flow, the rolling on VCAM-1 and transendothelial migration of NKT cells. Moreover, over-expression of high levels of CXCR4 on the YT NK cell line enables them to migrate in response to CXCL12. This study therefore suggests an important role for CXCR4 levels of expression and for VLA-4 in regulating the accumulation of NKT cells in the BM.
Eur J Immunol. 2004 May;34(5):1333-41

Gal I, Sadetzki S, Gershoni-Baruch R, Oberman B, Carp H, Papa MZ, Diestelman-Menachem T, Eisenberg-Barzilai S, Friedman E.
Offspring gender ratio and the rate of recurrent spontaneous miscarriages in jewish women at high risk for breast/ovarian cancer.
BRCA1/BRCA2 germline mutations are associated with an increased breast/ovarian cancer risk. Offspring gender ratios may be skewed against male births in BRCA1 mutation carriers. In addition, the lack of viable homozygous BRCA1/BRCA2-mutation carriers implies that recurrent miscarriages may be associated with homozygous fetuses. Jewish Israeli high-risk women who were tested for being carriers of the predominant BRCA1/BRCA2 mutations in Jewish high-risk families were analyzed for the sex of offspring and the rate of spontaneous miscarriages. Overall, 817 women participated: 393 BRCA1/BRCA2-mutation carriers (229 with breast/ovarian cancer) and 424 high-risk noncarriers (208 with breast/ovarian cancer). No differences between the male-to-female offspring ratios of all study groups were noted. Among mutation carriers, the offspring male-to-female ratio was 0.97 (444 : 460), and among mutation carriers with cancer it was 0.92 (262 : 284). Similarly, no

offspring gender skewing was noted among high-risk noncarriers, regardless of health status. The rates of three or more spontaneous miscarriages among participants with at least one live birth were 4.37% (15/343) among mutation carriers and 3% (12/401) among high-risk women (P = not significant). In conclusion, the offspring gender ratio is similar in high-risk Jewish families and in the general population. The issue of the rate of recurrent miscarriages in high-risk Jewish women is unresolved.
Am J Hum Genet. 2004 Jun;74(6):1270-5

Gerecht-Nir S, Dazard JE, Golan-Mashiach M, Osenberg S, Botvinnik A, Amariglio N, Domany E, Rechavi G, Givol D, Itskovitz-Eldor J.
Vascular gene expression and phenotypic correlation during differentiation of human embryonic stem cells.
The study of the cascade of events of induction and sequential gene activation that takes place during human embryonic development is hindered by the unavailability of postimplantation embryos at different stages of development. Spontaneous differentiation of human embryonic stem cells (hESCs) can occur by means of the formation of embryoid bodies (EBs), which resemble certain aspects of early embryos to some extent. Embryonic vascular formation, vasculogenesis, is a sequential process that involves complex regulatory cascades. In this study, changes of gene expression along the development of human EBs for 4 weeks were studied by large-scale gene screening. Two main clusters were identified-one of down-regulated genes such as POU5, NANOG, TDGF1/Cripto (TDGF, teratocarcinoma-derived growth factor-1), LIN28, CD24, TERF1 (telomeric repeat binding factor-1), LEFTB (left-right determination, factor B), and a second of up-regulated genes such as TWIST, WNT5A, WT1, AFP, ALB, NCAM1. Focusing on the vascular system development, genes known to be involved in vasculogenesis and angiogenesis were explored. Up-regulated genes include vasculogenic growth factors such as VEGFA, VEGFC, FIGF (VEGFD), ANG1, ANG2, TGFbeta3, and PDGFB, as well as the related receptors FLT1, FLT4, PDGFRB, TGFbetaR2, and TGFbetaR3, other markers such as CD34, VCAM1, PECAM1, VE-CAD, and transcription factors TAL1, GATA2, and GATA3. The reproducibility of the array data was verified independently and illustrated that many genes known to be involved in vascular development are activated during the differentiation of hESCs in culture. Hence, the analysis of the vascular system can be extended to other differentiation pathways, allocating human EBs as an in vitro model to study early human development.
Dev Dyn. 2004 Dec 21;232(2):487-497

Gnainsky Y, Spira G, Paizi M, Bruck R, Nagler A, Abu-Amara SN, Geiger B, Genina O, Monsonego-Ornan E, Pines M.
Halofuginone, an inhibitor of collagen synthesis by rat stellate cells, stimulates insulin-like growth factor binding protein-1 synthesis by hepatocytes.
BACKGROUND/AIMS: Halofuginone, an inhibitor of collagen synthesis, prevented and caused resolution of established hepatic fibrosis. A genomic approach in vivo was used to search for additional genes responsible for halofuginone mode of action. METHODS: Fibrosis was induced in rats by thioacetamide (TAA) and evaluated by collagen type I gene expression and the levels of collagen, tissue inhibitors of metalloproteinases-2 and smooth-muscle actin. Halofuginone was given in the diet. cDNA from liver biopsies was hybridized on Atlas arrays comprising of 588 genes. The results were confirmed by Northern blots and in situ hybridization. RESULTS: Insulin-like growth factor binding protein-1 (IGFBP-1) was one of the 13 genes

differentially expressed in the fibrotic liver after halofuginone treatment. After 2 and 4 weeks, halofuginone prevented the TAA-induced down-regulation of IGFBP-1 gene expression. Halofuginone also prevented the TAA-dependent changes in IGFBP-3 gene expression. Halofuginone affected IGFBP-1 synthesis in rat hepatocytes and cells of hepatocyte origin and caused time- and dose-dependent increases in the IGFBP-1 gene expression and synthesis by HepG2 cells. The IGFBP-1 secreted by HepG2-inhibited stellate cell motility. CONCLUSIONS: Halofuginone is an anti-fibrotic drug that inhibits collagen synthesis by stellate cells and preventing alteration in the synthesis of IGFBPs by hepatic cells.
J Hepatol. 2004 Feb;40(2):269-77.

Greenberger S, Shaish A, Varda-Bloom N, Levanon K, Breitbart E, Goldberg I, Barshack I, Hodish I, Yaacov N, Bangio L, Goncharov T, Wallach D, Harats D.

Transcription-controlled gene therapy against tumor angiogenesis.

A major drawback of current approaches to antiangiogenic gene therapy is the lack of tissue-specific targeting. The aim of this work was to trigger endothelial cell-specific apoptosis, using adenoviral vector-mediated delivery of a chimeric death receptor derived from the modified endothelium-specific pre-proendothelin-1 (PPE-1) promoter. In the present study, we constructed an adenovirus-based vector that targets tumor angiogenesis. Transcriptional control was achieved by use of a modified endothelium-specific promoter. Expression of a chimeric death receptor, composed of Fas and TNF receptor 1, resulted in specific apoptosis of endothelial cells in vitro and sensitization of cells to the proapoptotic effect of TNF-alpha. The antitumoral activity of the vectors was assayed in two mouse models. In the model of B16 melanoma, a single systemic injection of virus to the tail vein caused growth retardation of tumor and reduction of tumor mass with central tumor necrosis. When the Lewis lung carcinoma lung-metastasis model was applied, i.v. injection of vector resulted in reduction of lung-metastasis mass, via an antiangiogenic mechanism. Moreover, by application of the PPE-1-based transcriptional control, a humoral immune response against the transgene was avoided. Collectively, these data provide evidence that transcriptionally controlled, angiogenesis-targeted gene therapy is feasible.
J Clin Invest. 2004 Apr;113(7):1017-24.

Grunblatt E, Mandel S, Jacob-Hirsch J, Zeligson S, Amariglo N, Rechavi G, Li J, Ravid R, Roggendorf W, Riederer P, Youdim MB.

Gene expression profiling of parkinsonian substantia nigra pars compacta; alterations in ubiquitin-proteasome, heat shock protein, iron and oxidative stress regulated proteins, cell adhesion/cellular matrix and vesicle trafficking genes.

Gene expression profiling of human substantia nigra pars compacta (SNpc) from Parkinson's disease (PD) patients, was examined employing high density microarrays. We identified alterations in the expression of 137 genes, with 68 down regulated and 69 up regulated. The down regulated genes belong to signal transduction, protein degradation (e.g. ubiquitin-proteasome subunits), dopaminergic transmission/metabolism, ion transport, protein modification/phosphorylation and energy pathways/glycolysis functional classes. Up-regulated genes, clustered mainly in biological processes involving cell adhesion/cytoskeleton, extracellular matrix

components, cell cycle, protein modification/phosphorylation, protein metabolism, transcription and inflammation/stress (e.g. key iron and oxygen sensor EGLN1). One major finding in the present study is the particular decreased expression of SKP1A, a member of the SCF (E3) ligase complex specifically in the substantia nigra (SN) of sporadic parkinsonian patients, which may lead to a wide impairment in the function of an entire repertoire of proteins subjected to regulatory ubiquitination. These findings reveal novel players in the neurodegenerative scenario and provide potential targets for the development of novel drug compounds.
J Neural Transm. 2004 Dec;111(12):1543-73

Gur G, Rubin C, Katz M, Amit I, Citri A, Nilsson J, Amariglio N, Henriksson R, Rechavi G, Hedman H, Wides R, Yarden Y.
LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation.
Kekkon proteins negatively regulate the epidermal growth factor receptor (EGFR) during oogenesis in Drosophila. Their structural relative in mammals, LRIG1, is a transmembrane protein whose inactivation in rodents promotes skin hyperplasia, suggesting involvement in EGFR regulation. We report upregulation of LRIG1 transcript and protein upon EGF stimulation, and physical association of the encoded protein with the four EGFR orthologs of mammals. Upregulation of LRIG1 is followed by enhanced ubiquitylation and degradation of EGFR. The underlying mechanism involves recruitment of c-Cbl, an E3 ubiquitin ligase that simultaneously ubiquitylates EGFR and LRIG1 and sorts them for degradation. We conclude that LRIG1 evolved in mammals as a feedback negative attenuator of signaling by receptor tyrosine kinases.
EMBO J. 2004 Aug 18;23(16):3270-81.

Gur H, Krauthgamer R, Bachar-Lustig E, Katchman H, Arbel-Goren R, Berrebi A, Klein T, Nagler A, Tabilio A, Martelli MF, Reisner Y.
Immune regulatory activity of CD34+ progenitor cells: evidence for a deletion based mechanism mediated by TNF-{alpha}.
Abstract Previous studies suggest that cells within the CD34(+) hematopoietic stem cell compartment are endowed with immune regulatory activity. Furthermore, it is possible to expand the human regulatory cells upon short-term culture of purified CD34(+) cells with an early-acting cytokine cocktail. We now show that addition of anti-CD28, anti-CD2, IL-2, anti-IL-10, or IL-12 to the bulk MLR cannot reverse the inhibitory activity of the CD34(+) cells, rulling out anergy based mechanisms, or mechanisms involving Th1-Th2 skewing. Furthermore, phenotyping of cells present after addition of CD34(+) cells to the bulk MLR, ruled out potential induction of plasmacytoid dendritic precursors, known to be endowed with regulatory activity. In contrast, the inhibitory activity of CD34(+) cells could be reversed by adding the caspase inhibitor BD-FMK to the bulk MLR, indicating a deletion based mechanism. The deletion can be inhibited by anti-TNF-alpha and not by anti-TGF-beta, suggesting a potential role for TNF-a in the regulatory activity of CD34(+) cells.
Blood. 2004 Oct 7

Hardan I, Rothman R, Gelibter A, Cohen N, Shimoni A, Sokolovsky M, Reichart M, Ishoev G, Amariglio N, Rechavi G, Nagler A, Trakhtenbrot L.
Determination of chromosome 13 status in bone marrow cells of patients with multiple myeloma using combined morphologic and fluorescence in situ

hybridization analysis.
Deletion of chromosome 13q is believed to be an adverse prognostic marker in patients with multiple myeloma (MM). Interphase fluorescence in situ hybridization (I-FISH) is the method of choice for detection of chromosome 13q deletion (del13q). However, I-FISH has high false-positive rates attributed to a low percentage of plasma cells (PC), which are responsible for MM, in bone marrow (BM) samples from MM patients. In an attempt to overcome this problem, combined morphologic and I-FISH analyses were performed by a unique system that allows rapid automatic scanning of a large number of cells with simultaneous determination of the lineage of specific cells carrying del13q. The percentage of PC with del13q in BM samples from 40 MM patients was calculated. In addition, we established a useful prognostic ratio defined as the number of PC with del13q divided by the number of non-PC with del13q (PDP/PDNP), which may help to precisely define the putative role of del13q in prediction response of MM patients to new therapeutic compounds. We suggest this technique as a novel sensitive and specific method for detection of del13q in a minor PC population of MM patients.
Exp Hematol. 2004 Mar;32(3):254-60

Hertzano R, Montcouquiol M, Rashi-Elkeles S, Elkon R, Yucel R, Frankel WN, Rechavi G, Moroy T, Friedman TB, Kelley MW, Avraham KB.
Transcription profiling of inner ears from Pou4f3(ddl/ddl) identifies Gfi1 as a target of the Pou4f3 deafness gene.
Pou4f3 (Brn3.1, Brn3c) is a class IV POU domain transcription factor that has a central function in the development of all hair cells in the human and mouse inner ear sensory epithelia. A mutation of POU4F3 underlies human autosomal dominant non-syndromic progressive hearing loss DFNA15. Through a comparison of inner ear gene expression profiles of E16.5 wild-type and Pou4f3 mutant deaf mice using a high density oligonucleotide microarray, we identified the gene encoding growth factor independence 1 (Gfi1) as a likely in vivo target gene regulated by Pou4f3. To validate this result, we performed semi-quantitative RT-PCR and in situ hybridizations for Gfi1 on wild-type and Pou4f3 mutant mice. Our results demonstrate that a deficiency of Pou4f3 leads to a statistically significant reduction in Gfi1 expression levels and that the dynamics of Gfi1 mRNA abundance closely follow the pattern of expression for Pou4f3. To examine the role of Gfi1 in the pathogenesis of Pou4f3-related deafness, we performed comparative analyses of the embryonic inner ears of Pou4f3 and Gfi1 mouse mutants using immunohistochemistry and scanning electron microscopy. The loss of Gfi1 results in outer hair cell degeneration, which appears comparable to that observed in Pou4f3 mutants. These results identify Gfi1 as the first downstream target of a hair cell specific transcription factor and suggest that outer hair cell degeneration in Pou4f3 mutants is largely or entirely a result of the loss of expression of Gfi1.
Hum Mol Genet. 2004 Sep 15;13(18):2143-53.

Inbal A, Lubetsky A, Shimoni A, Dardik R, Sela BA, Eskaraev R, Levi I, Tov NS, Nagler A.
Assessment of the coagulation profile in hemato-oncological patients receiving ATG-based conditioning treatment for allogeneic stem cell transplantation.
Antithymocyte globulin (ATG) is increasingly used in pre-allogeneic stem cell transplantation (allo-SCT) conditioning regimens to prevent graft rejection and graft-

versus-host disease. However, ATG was also found to be associated with increased incidence of thrombosis during organ transplantation. In the present study, we tested the coagulation status of 21 patients with hematologic malignancies undergoing allo-SCT who received ATG-based (11 patients) or non-ATG-based (10) conditioning treatment. We assessed several thrombophilia markers as well as circulating total and endothelial microparticles (TMP/EMP) and soluble CD40 ligand (CD40L). No significant difference in the mean values of prothrombin time, partial thromboplastin time, fibrinogen, antithrombin, protein C, protein S, thrombin-antithrombin III complex, homocysteine levels, prevalence of genetic thrombophilia markers and levels of EMP, TMP or CD40L was observed between the ATG-treated and ATG-untreated patients, as well as before and after conditioning in each group separately. Platelet counts decreased significantly in ATG-treated patients; however, this decrease was not associated with clinical or laboratory evidence of disseminated intravascular coagulation. No patient developed thromboembolic event or veno-occlusive liver disease. Our results suggest that allo-SCT is not associated with increased hypercoagulability and addition of ATG to conditioning regimen has no significant procoagulant effect.
Bone Marrow Transplant. 2004 Sep;34(5):459-63.

Israeli O, Gotlieb WH, Friedman E, Korach J, Friedman E, Goldman B, Zeltser A, Ben-Baruch G, Rienstein S, Aviram-Goldring A.
Genomic analyses of primary and metastatic serous epithelial ovarian cancer.
Epithelial ovarian cancer is the most lethal gynecologic malignancy in the western world. In 75% of patients, peritoneal metastases are found at the time of primary surgery. However, the genetic events leading to the development of ovarian tumors and to the genetic progression toward metastasis remain unclear. To gain insight into this issue, the types and patterns of DNA copy number changes were compared between primary ovarian tumors and their respective metastases by using comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). The genetic alterations (deletions and amplifications) detected by CGH were similar in the primary tumors and in their respective metastases. Moreover, the FISH results show a similar pattern of chromosomal abnormalities. Our results imply that the major gross genetic changes in ovarian cancer take place in the primary tumor, and the additional genetic changes that may occur in the metastases are not detectable by CGH.
Cancer Genet Cytogenet. 2004 Oct 1;154(1):16-21

Izraeli S, Waldman D.
Minimal residual disease in childhood acute lymphoblastic leukemia: current status and challenges.
The pace of disappearance of leukemic blasts in response to therapy has long been recognized as the most important prognostic factor in childhood acute lymphoblastic leukemia (ALL). Recent technological advancements enable detection of submicroscopic leukemic cells. The extent of reduction in the level of minimal residual disease (MRD) during the first phase of therapy can be exploited for improved risk classification of children with ALL. Current prospective studies test the hypothesis that tailoring treatment to the level of MRD will improve patients' outcome.
Acta Haematol. 2004;112(1-2):34-9

Kario E, Marmor MD, Adamsky K, Citri A, Amit I, Amariglio N, Rechavi G, Yarden Y.

Suppressors of cytokine signaling 4 and 5 regulate epidermal growth factor receptor signaling.

Suppressors of cytokine signaling (SOCS) are SH2-containing proteins originally identified as negative regulators of cytokine signaling. Accumulating evidence indicates a role for SOCS proteins in the regulation of additional signaling pathways, including receptor tyrosine kinases (RTKs). Notably, SOCS36E, the Drosophila ortholog of mammalian SOCS5, was recently implicated as a negative regulator of DER, the Drosophila ortholog of EGFR. In this study, we aimed at characterizing the role of SOCS5 in the negative regulation of EGFR. We show here that expression of SOCS5 and its closest homolog SOCS4 is elevated in cells following treatment with EGF, similar to several negative feedback regulators of EGFR whose expression is up-regulated upon receptor activation. The expression of SOCS5 led to a marked reduction in EGFR expression levels, by promoting EGFR degradation. The reduction in EGFR levels and EGF-induced signaling in SOCS5-expressing cells requires both the SH2 and SOCS box (SB) domains of SOCS5. Interestingly, EGFR is degraded by SOCS5 prior to EGF treatment, in a ligand- and c-Cbl-independent manner. SOCS5 can associate with EGFR and can also bind the ElonginBC protein complex via its SB, which may recruit an E3 ubiquitin ligase to promote EGFR degradation. Thus, we have characterized a novel function for SOCS5 in regulating EGFR, and discuss its potential role in controling EGFR homeostasis.

J Biol Chem. 2004 Dec 7; [Epub ahead of print]

Katz D, Segal A, Alberton Y, Jurim O, Reissman P, Catane R, Cherny NI.

Neoadjuvant imatinib for unresectable gastrointestinal stromal tumor.

We have evaluated the feasibility of the use of neoadjuvant imatinib mesylate in the management of unresectable localized gastrointestinal stromal tumors. In a pilot experience, two patients with unresectable gastrointestinal tumors were treated with neoadjuvant imatinib. Their treatment course and surgical outcomes are described. In both cases, the patient attained sufficient tumor regression to enable complete resection of tumor. We conclude that in the management of unresectable gastrointestinal stromal tumors, neoadjuvant administration of imatinib may facilitate sufficient tumor regression to facilitate subsequent tumor resection with curative intent.

Anticancer Drugs. 2004 Jul;15(6):599-602

Kaufman Y, Drori S, Cole PD, Kamen BA, Sirota J, Ifergan I, Arush MW, Elhasid R, Sahar D, Kaspers GJ, Jansen G, Matherly LH, Rechavi G, Toren A, Assaraf YG.

Reduced folate carrier mutations are not the mechanism underlying methotrexate resistance in childhood acute lymphoblastic leukemia.

BACKGROUND: Although the majority of children with acute lymphoblastic leukemia (ALL) are cured with combination chemotherapy containing methotrexate (MTX), drug resistance contributes to treatment failure for a substantial fraction of patients. The primary transporter for folates and MTX is the reduced folate carrier (RFC). Impaired drug transport is a documented mechanism of MTX resistance in patients with ALL; however, to the authors' knowledge it is not known whether inactivating RFC mutations are a contributing factor. METHODS: The authors devised a genomic polymerase chain reaction-single strand conformational

polymorphism assay followed by sequencing and screened the entire RFC coding region for sequence alterations in DNA from 246 leukemia specimens from patients with diverse ethnic variation, 24 at the time of recurrence and the rest at the time of diagnosis. This cohort was comprised of 203 B-precursor ALL specimens (82.5%), 32 T-lineage ALL specimens (13%), and 11 acute myeloblastic leukemia specimens (4.5%). RESULTS: Of 246 DNA samples, only 3 diagnosis B-precursor ALL specimens (1.2%) were found to harbor alterations in the RFC gene, including heterozygous single nucleotide changes resulting in D56H and D522N substitutions in the first extracellular loop and the C-terminus of this transporter, respectively. The third sample had a sequence alteration in exon 3 that could not be identified because of the lack of availability of DNA. CONCLUSIONS: Whereas inactivating RFC mutations are a frequent mechanism of MTX resistance in human leukemia cell lines and in patients with osteosarcoma, they are not common and do not appear to play any significant role in intrinsic or acquired resistance to MTX in childhood leukemia. This is the first study of RFC mutations in multiple pediatric leukemia specimens
Cancer. 2004 Feb 15;100(4):773-82

Keidan I, Perel A, Shabtai EL, Pfeffer RM.
Children undergoing repeated exposures for radiation therapy do not develop tolerance to propofol: clinical and bispectral index data.
BACKGROUND: The purpose of this study was to apply clinical criteria and Bispectral Index monitor data for evaluating the development of tolerance to propofol in children undergoing repeated drug exposure. METHODS: Children undergoing multiple sessions of radiation therapy during anesthesia for various malignancies were given a predetermined dose of propofol at each session. Heart rate, blood pressure, oxygen saturation, respiratory rate, requirement of additional propofol, and time to emergence and discharge were recorded. The Bispectral Index was monitored continuously, and parameters were extracted and averaged for each week of therapy. RESULTS: Fifteen children (aged 2.5-10 yr) were treated for an average of 5 weeks (24 +/- 6 sessions). There were no significant differences in physiologic parameters or requirements of additional propofol between the weeks of treatment. Bispectral Index data analysis showed that although a nonlinear change with time for each parameter could not be rejected, the differences between the first and last intervals were nonsignificant. CONCLUSIONS: Overall changes with time resulted from random fluctuations without a consistent trend. Combined with clinical data, Bispectral Index parameters showed that tolerance to propofol does not develop in children undergoing repeated exposures to the drug during radiation therapy.
Anesthesiology. 2004 Feb;100(2):251-4.

Kroger N, Schilling G, Einsele H, Liebisch P, Shimoni A, Nagler A, Perez-Simon JA, San Miguel JF, Kiehl M, Fauser A, Schwerdtfeger R, Wandt H, Sayer HG, Myint H, Klingemann H, Zabelina T, Dierlamm J, Hinke A, Zander AR.
Deletion of chromosome band 13q14 as detected by fluorescence in situ hybridization is a prognostic factor in patients with multiple myeloma who are receiving allogeneic dose-reduced stem cell transplantation.
We investigated in a retrospective multicenter study the impact of chromosome arm 13q deletion (13q-) as detected by fluorescence in situ hybridization (FISH) on outcome after dose-reduced allografting in patients with multiple myeloma. In 68 of 140 patients, data on chromosome 13q status were available. Most patients included had advanced myeloma. At 2 years, patients with 13q deletion (n = 31) had a shorter

event-free (18% vs 42%; P =.05) and overall survival (18% vs 67%; P =.03) than patients without 13q- (n = 37). Patients with 13q- experienced a higher relapse rate (77% vs 44%; P <.001) but a similar incidence of transplantation-related mortality at one year (24% vs 18%). In a multivariate analysis, 13q- remained a significant risk factor for a higher relapse rate (hazard ratio [HR], 3.28; 95% confidence interval [CI], 1.31-8.24; P =.01) and a shorter event-free survival (HR, 1.94; 95% CI, 1.03-3.67; P =.04). Concerning overall survival, 2 or more cycles of prior high-dose chemotherapy were associated with a significantly higher probability of death (HR, 2.48; 95% CI, 1.19-5.17; P =.02), while patients with deletion 13q had a nearly 2 times higher risk of death (HR, 1.94; 95% CI, 0.95-3.98; P =.07) after dose-reduced allogeneic stem cell transplantation.
Blood. 2004 Jun 1;103(11):4056-61

Kroger N, Shimoni A, Zagrivnaja M, Ayuk F, Lioznov M, Schieder H, Renges H, Fehse B, Zabelina T, Nagler A, Zander AR.
Low-dose thalidomide and donor lymphocyte infusion as adoptive immunotherapy after allogeneic stem cell transplantation in patients with multiple myeloma.
To improve the antimyeloma effect of donor lymphocyte infusion (DLI) after allogeneic stem cell transplantation in multiple myeloma, we investigated in a phase 1/2 study the effect of low-dose thalidomide (100 mg) followed by DLI in 18 patients with progressive disease or residual disease and prior ineffective DLI after allografting. The overall response rate was 67%, including 22% complete remission. Major toxicity of thalidomide was weakness grade I/II (68%) and peripheral neuropathy grade I/II (28%). Only 2 patients experienced mild grade I acute graft versus host disease (aGvHD) of the skin, while no grades II to IV aGvHD was seen. De novo limited chronic GvHD (cGvHD) was seen in 2 patients (11%). The 2-year estimated overall and progression-free survival were 100% and 84%, respectively. Adoptive immunotherapy with low-dose thalidomide and DLI induces a strong antimyeloma effect with low incidence of graft versus host disease
Blood. 2004 Nov 15;104(10):3361-3.

Kundel Y, Pfeffer R, Lauffer M, Ramon J, Catane R, Symon Z.
Salvage prostatic fossa radiation therapy for biochemical failure after radical prostatectomy: the Sheba experience.
BACKGROUND: The role of prostatic fossa radiation as salvage therapy in the setting of a rising prostate-specific antigen following radical prostatectomy is not well defined. OBJECTIVES: To study the efficacy and safety of pelvic and prostatic fossa radiation therapy following radical prostatectomy for adenocarcinoma. METHODS: A retrospective review of the charts of 1,050 patients treated at the Sheba Medical Center for prostate cancer between 1990 and 2002 identified 48 patients who received post-prostatectomy pelvic and prostatic fossa radiotherapy for biochemical failure. Two patients were classified as T1, T2A-9, T2B-19, T3A-7 and T3B-11. Gleason score was 2-4 in 9 patients, 5-6 in 22 patients, 7 in 10 patients and 8-10 in 7 patients. Positive surgical margins were noted in 28 patients (58%) of whom 18 had single and 10 had multiple positive margins. Radiation was delivered with 6 mV photons using a four-field box to the pelvis followed by two lateral arcs to the prostatic fossa. RESULTS: At a median follow-up of 34.3 months (25th, 75th) (14.7, 51.3) since radiation therapy, 32 patients (66%) are free of disease or biochemical failure. Exploratory analysis revealed that a pre-radiation PSA less than 2 ng/ml was

associated with a failure rate of 24% compared with 66% in patients with a pre-radiation PSA greater than 2 ng/ml (chi-square P < 0.006). CONCLUSIONS: For patients with biochemical failure following radical prostatectomy early salvage radiation therapy is an effective and safe treatment option
Isr Med Assoc J. 2004 Jun;6(6):329-31

Levanon EY, Eisenberg E, Yelin R, Nemzer S, Hallegger M, Shemesh R, Fligelman ZY, Shoshan A, Pollock SR, Sztybel D, Olshansky M, Rechavi G, Jantsch MF.

Systematic identification of abundant A-to-I editing sites in the human transcriptome.

RNA editing by members of the ADAR (adenosine deaminases acting on RNA) family leads to site-specific conversion of adenosine to inosine (A-to-I) in precursor messenger RNAs. Editing by ADARs is believed to occur in all metazoa, and is essential for mammalian development. Currently, only a limited number of human ADAR substrates are known, whereas indirect evidence suggests a substantial fraction of all pre-mRNAs being affected. Here we describe a computational search for ADAR editing sites in the human transcriptome, using millions of available expressed sequences. We mapped 12,723 A-to-I editing sites in 1,637 different genes, with an estimated accuracy of 95%, raising the number of known editing sites by two orders of magnitude. We experimentally validated our method by verifying the occurrence of editing in 26 novel substrates. A-to-I editing in humans primarily occurs in noncoding regions of the RNA, typically in Alu repeats. Analysis of the large set of editing sites indicates the role of editing in controlling dsRNA stability
Nat Biotechnol. 2004 Aug;22(8):1001-5.

Leventhal A, Karsenty E, Sadetzki S.

Cellular phones and public health

BACKGROUND: The increased use of mobile cellular phone by the public is associated with a wave of contradictory reports about the possible health effects, due to the exposure of the users to electromagnetic non-ionizing radiation. AIMS: This article reviews the state of the art of the present knowledge concerning the biological and medical effects of exposure to cellular phones, with an emphasis on its possible carcinogenic effect. RESULTS: Health conditions, which have been ascribed to the use of mobile phones mainly include some types of cancer and changes of brain activity. However, the balance of evidence from available studies has not yet supported these claims. Following the recommendation of special international expert committees, the IARC (International Association for Research on Cancer) is conducting a multi-center study to determine the possible effect of cellular phone use on brain and salivary gland tumors. Israel is one of the participants of this study. The only established health effect associated with the use of such technology is an increased risk for road accidents, unrelated to the amount of radiation emitted by phone. CONCLUSIONS: The challenge posed by this new technology to health authorities all over the world has lead to the definition of a new principle, the so-called "prudent avoidance", used as guidelines for the definition of an adequate public health policy. The public policy in Israel has used the prudent avoidance principles, while awaiting the results of the multi-national epidemiological studies
Harefuah. 2004 Aug;143(8):614-8, 620

Levitt ML, Kassem B, Gooding WE, Miketic LM, Landreneau RJ, Ferson PF, Keenan R, Yousem SA, Lindberg CA, Trenn MR, Ponas RS, Tarasoff P, Sabatine JM, Friberg D, Whiteside TL.

Phase I study of gemcitabine given weekly as a short infusion for non-small cell lung cancer: results and possible immune system-related mechanisms.

PURPOSE: To define the maximum tolerated dose (MTD) and the nature of the toxicities associated with gemcitabine given as a short infusion to patients with non-small cell lung cancer (NSCLC). Secondary objectives were to monitor immunologic response, clinical response, and survival. PATIENTS AND METHODS: Thirty-two patients diagnosed with advanced inoperable NSCLC and performance status of 0 or 1 participated in this study. Patients consisted of 22 males and 10 females whose median age was 62 years (range 32-79). Gemcitabine was administered as a 30 min infusion once weekly for 3 weeks followed by 1 week of rest. Patients were enrolled at six gemcitabine dose levels ranging from 1000 to 3500 mg/m2. Patients completed a median of four cycles (range 1-17). Responses were evaluated after every two cycles. RESULTS: Toxicity was evaluated in all 32 patients. The MTD was not reached as gemcitabine was well tolerated at all dose levels. Grade 4 toxicity occurred in three (9%) patients: pulmonary and lymphocytopenia in one patient each, and both neurocortical and cardiac in one patient. Grade 3 toxicity was found in a total of 20 (63%) patients: pulmonary in 10 (31%) patients; pain in 6 (19%) patients; liver toxicity in 6 (19%) patients; leukopenia and lymphocytopenia in 5 (16%) patients each; anemia, nausea, and cardiac toxicity in 3 (9%) patients each; proteinuria and infection in 2 (6%) patients each; and hemorrhage in 1 (3%) patient. Of the 29 patients evaluable for response, seven objective responses were achieved: six at the 2200 mg/m2 dose level and one at the 2800 mg/m2 dose level. The distribution of responses differed significantly by dose (P = 0.0124 by the exact chi-square test for independence). The overall response rate was 24.1% (95% CI, 10.3-43.5%). At 6 h post-infusion, there was a significant increase in spontaneous tumor necrosis factor (TNF) release and stimulated interleukin (IL)-2 production, and significant decreases in total white blood cell and lymphocyte counts (CD3+, CD8+, and CD16+ lymphocytes) and resting and stimulated superoxide production by formyl-methionyl-leucyl-phenylalanine (fMLP), phorbol myristate acetate, and opsonized zymosan (OPS-Z). At 24 h post-infusion, there were significant decreases in total lymphocyte count, lymphocyte subsets (CD3+, CD4-, CD8+, CD56+, CD19+), and in resting and stimulated superoxide production by fMLP and OPS-Z. There also appeared to be an association between the levels of spontaneous TNF release and the severity of both gastrointestinal (GI) and pulmonary toxicities. CONCLUSION: Gemcitabine given as a short infusion was well tolerated at the dose levels of 1000-3500 mg/m2. The MTD was not reached. Toxicities appeared to be cumulative with multiple cycles. Gemcitabine appears to have activity against NSCLC. Although there was a differential dose-response rate among dose levels, increasing the gemcitabine dose beyond 2200mg/m2 did not show increased clinical response. Gemcitabine appears to modulate the immune response, which may in turn mediate both response and toxicity, although no statistically significant correlation between immune and clinical response was detected.

Lung Cancer. 2004 Mar;43(3):335-44

Lidar Z, Mardor Y, Jonas T, Pfeffer R, Faibel M, Nass D, Hadani M, Ram Z.

Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study.

OBJECT: A minority of patients with recurrent glioblastomas multiforme (GBMs) responds to systemic chemotherapy. The authors investigated the safety and efficacy of intratumoral convection-enhanced delivery (CED) of paclitaxel in patients harboring histologically confirmed recurrent GBMs and anaplastic astrocytomas. METHODS: Fifteen patients received a total of 20 cycles of intratumoral CED of paclitaxel. The patients were observed daily by performing diffusion-weighted (DW) magnetic resonance (MR) imaging to assess the convective process and routine diagnostic MR imaging to identify the tumor response. Effective convection was determined by the progression of the hyperintense signal within the tumor on DW MR images, which corresponded to a subsequent lytic tumor response displayed on conventional MR images. Of the 15 patients, five complete responses and six partial responses were observed, giving a response rate of 73%. The antitumor effect was confirmed by one biopsy and three en bloc resections of tumors, which showed a complete response, and by one tumor resection, which demonstrated a partial response. Lack of convection and a poor tumor response was associated with leakage of the convected drug into the subarachnoid space, ventricles, and cavities formed by previous resections, and was seen in tumors containing widespread necrosis. Complications included transient chemical meningitis in six patients, infectious complications in three patients, and transient neurological deterioration in four patients (presumably due to increased peritumoral edema). CONCLUSIONS: On the basis of our data we suggest that CED of paclitaxel in patients with recurrent malignant gliomas is associated with a high antitumor response rate, although it is associated with a significant incidence of treatment-associated complications. Diffusion-weighted MR images may be used to predict a response by demonstrating the extent of convection during treatment. Optimization of this therapeutic approach to enhance its efficacy and reduce its toxicity should be explored further.
J Neurosurg. 2004 Mar;100(3):472-9.

Lotem M, Shiloni E, Pappo I, Drize O, Hamburger T, Weitzen R, Isacson R, Kaduri L, Merims S, Frankenburg S, Peretz T.

Interleukin-2 improves tumour response to DNP-modified autologous vaccine for the treatment of metastatic malignant melanoma.

This paper is a report of response rate (RR) and survival of 34 metastatic melanoma patients who received a dinitrophenyl (DNP)-modified autologous melanoma cell vaccine. In all, 27 patients started the vaccine as a primary treatment for metastatic melanoma and seven started it as an adjuvant, with no evidence of disease at the time, but had developed new metastases. Interleukin-2 (IL-2) was administered in 24 out of the 34 patients: 19 who progressed on vaccine alone and five who had the combination from start. Interleukin-2 was administered in the intravenous, bolus high-dose regimen (seven patients) or as subcutaneous (s.c.) low-dose treatment (17). Overall response for the entire group was 35% (12 patients out of 34), 12% having a complete response (CR) and 23% a partial response (PR). However, only two patients had tumour responses while on the vaccine alone, whereas the other 10 demonstrated objective tumour regression following the combination with IL-2 (two CR, eight PR), lasting for a median duration of 6 months (range 3-50 months). Of the 12 responding patients, 11 attained strong skin reactivity to the s.c. injection of irradiated, unmodified autologous melanoma cells. None of the patients with a negative reactivity experienced any tumour response. Patients with positive skin reactions survived longer (median survival - 54 months). The results suggest enhanced RRs to the combination of IL-2 and autologous melanoma vaccine. Skin reactivity to

unmodified autologous melanoma cells may be a predictor of response and improved survival, and therefore a criterion for further pursuing of immunotherapeutic strategies.
Br J Cancer. 2004 Feb 23;90(4):773-80

Magal-Vardi O, Laor N, Toren A, Strauss L, Wolmer L, Bielorai B, Rechavi G, Toren P.

Psychiatric morbidity and quality of life in children with malignancies and their parents.

Recent improvements in prognosis necessitate considering the emotional responses of children with malignant diseases and of their parents. This prospective study assessed 20 children and adolescents and their 36 parents within 2 weeks of diagnosis and after 1 and 6 months. Fifty-three percent exhibited moderate to severe posttraumatic symptoms right after diagnosis that decreased significantly after 1 month. Children with high-risk disease reported the most severe symptoms. Unexpectedly, children with low-risk disease exhibited more severe symptoms than those with moderate risk. Depressive symptoms decreased significantly during the period, but anxiety symptoms did not. Moreover, quality of life did not change. Twenty percent of parents exhibited posttraumatic symptoms on initial evaluation. Mothers' symptoms did not change, but fathers' symptoms decreased with those of their children. Several procedures and experiences were identified as causes of traumatic stress responses
J Nerv Ment Dis. 2004 Dec;192(12):872-5.

Mardor Y, Roth Y, Ochershvilli A, Spiegelmann R, Tichler T, Daniels D, Maier SE, Nissim O, Ram Z, Baram J, Orenstein A, Pfeffer R.

Pretreatment prediction of brain tumors' response to radiation therapy using high b-value diffusion-weighted MRI.

Diffusion-weighted magnetic resonance imaging (DWMRI) is sensitive to tissues' biophysical characteristics, including apparent diffusion coefficients (ADCs) and volume fractions of water in different populations. In this work, we evaluate the clinical efficacy of DWMRI and high diffusion-weighted magnetic resonance imaging (HDWMRI), acquired up to b = 4000 sec/mm(2) to amplify sensitivity to water diffusion properties, in pretreatment prediction of brain tumors' response to radiotherapy. Twelve patients with 20 brain lesions were studied. Six ring-enhancing lesions were excluded due to their distinct diffusion characteristics. Conventional and DWMRI were acquired on a 0.5-T MRI. Response to therapy was determined from relative changes in tumor volumes calculated from contrast-enhanced T1-weighted MRI, acquired before and a mean of 46 days after beginning therapy. ADCs and a diffusion index, R(D), reflecting tissue viability based on water diffusion were calculated from DWMRIs. Pretreatment values of ADC and R(D) were found to correlate significantly with later tumor response/nonresponse ($r = 0.76$, $P < .002$ and $r = 0.77$, $P < .001$). This correlation implies that tumors with low pretreatment diffusion values, indicating high viability, will respond better to radiotherapy than tumors with high diffusion values, indicating necrosis. These results demonstrate the feasibility of using DWMRI for pretreatment prediction of response to therapy in patients with brain tumors undergoing radiotherapy.
Neoplasia. 2004 Mar-Apr;6(2):136-42

Merimsky O, Gez E, Weitzen R, Nehushtan H, Rubinov R, Hayat H, Peretz T, Ben-Shahar M, Biran H, Katsenelson R, Mermershtein V, Loven D, Karminsky N, Neumann A, Matcejevsky D, Inbar M.

Targeting pulmonary metastases of renal cell carcinoma by inhalation of interleukin-2.

INTRODUCTION: Pulmonary metastases of renal cell carcinoma (RCC) are associated with poor prognosis. Inhalation therapy with interleukin-2 (IL-2) is thus an appealing method for palliation. This multicenter study summarizes the national experience of IL-2 inhalation in patients with lung metastases of RCC. PATIENTS AND METHODS: Forty patients (median, 66.5 years of age) with radiologically documented progressing pulmonary metastases were enrolled. All patients had to be able to comply with inhalation technique, and were not candidates for other treatment options. Twenty-eight patients were systemic treatment-naive. The protocol included three daily inhalations of IL-2 to a total dose of 18 MU. Treatment had to be continued until one of the following occurred: progression; a complete response; a life threatening toxicity; or patient refusal. Response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) system. RESULTS: The disease-control rate reached 57.5%, with a partial response rate of 2.5% and a disease stabilization rate of 55%. Median time to progression was 8.7 months. The main side-effects were cough and weakness. CONCLUSIONS: Inhalation of IL-2 for the treatment of pulmonary metastases in RCC is feasible, tolerable and beneficial in controlling progressive disease for considerable periods of time. The definition of response of biological therapy may need to be re-assessed and modified: stable disease should be regarded as a favorable response.

Ann Oncol. 2004 Apr;15(4):610-2.

Nagler A, Korenstein-Ilan A, Amiel A, Avivi L.

Granulocyte colony-stimulating factor generates epigenetic and genetic alterations in lymphocytes of normal volunteer donors of stem cells.

OBJECTIVE: Because the effect of granulocyte colony-stimulating factor (G-CSF), which is widely used for allogeneic stem cell transplantation, on DNA function and stability has not yet been unequivocally elucidated, the aim of this study was to determine whether G-CSF leads to epigenetic and/or genetic modifications. MATERIALS AND METHODS: Molecular cytogenetic techniques based on fluorescence in situ hybridization technology were used. RESULTS: Lymphocytes of G-CSF mobilized donors displayed epigenetic (altered replication timing of alleles) and genetic (aneuploidy) alterations similar to those observed in lymphocytes of cancer patients. Specifically, in the donors' lymphocytes, biallelically expressed genes (TP53 and AML1) and a repetitive noncoding DNA sequence associated with chromosome segregation (CEN17) showed loss of synchrony in allelic replication timing (allele-specific replication). Each displayed a highly asynchronous pattern of allelic replication similar to that characterizing monoallelic expressed genes. This non-locus-specific epigenetic phenomenon, which also affects DNA sequences associated with chromosome segregation, was accompanied by aneuploidy. Although the loss of replication synchrony in the lymphocytes of G-CSF mobilized donors was a transient epigenetic modification, aneuploidy remained unchanged. The G-CSF effect also was observed after G-CSF administration in vitro. 5-Azacytidine, a DNA methylation blocking agent, inhibited G-CSF in vitro induction of allele-specific replication. CONCLUSION: G-CSF, probably via changes in DNA methylation capacity, leads to cancer-characteristic DNA modifications in lymphocytes of normal

mobilized donors.
Exp Hematol. 2004 Jan;32(1):122-30.

Nagler A, Ohana M, Shibolet O, Shapira MY, Alper R, Vlodavsky I, Pines M, Ilan Y. Suppression of hepatocellular carcinoma growth in mice by the alkaloid coccidiostat halofuginone.
Halofuginone, a widely used alkaloid coccidiostat, is a potent inhibitor of collagen alpha 1 (I) and matrix metalloproteinase 2 gene expression. Halofuginone also suppresses extracellular matrix deposition and fibroblast proliferation. It was recently shown to be effective in suppression of bladder carcinoma and glioma. This study sought to evaluate the effect of treatment with halofuginone on growth of hepatocellular carcinoma (HCC) in mice. Athymic Balb/c mice were injected subcutaneously with 10(7) human hepatoma cells (Hep3B), followed by treatment with halofuginone administered in the diet (750 microg/kg) starting on day 3, before tumour innoculation. The control group was received a normal diet. Mice were followed for survival, tumour volume and serum alpha-fetoprotein (alpha FP). The mechanism of the anti-tumour effect of halofuginone was determined in vitro by assessing tumour cell growth, and by measuring the serum concentrations of interferon-gamma (IFN gamma) and interleukin 2 (IL2). Halofuginone treatment induced almost complete tumour suppression in treated mice. Mortality rates were 10% and 50%, in halofuginone-treated and control mice, respectively (P<0.001). No visible tumour was observed in treated mice, as compared with a 364 mm3 tumour in control mice. Serum alpha FP were 0.1 and 212 ng/ml in treated and control mice, respectively (P<0.005). Halofuginone significantly inhibited HCC proliferation in vitro. Maximal inhibition of 64% of tumour cell growth was observed at a concentration of 10(-8) M. The anti-tumour effect was mediated via a significant increase in IFN gamma and IL2 (90 vs. 35, and 210 vs. 34 pg/ml in treated and control groups, respectively, P<0.005). Treatment with halofuginone effectively suppressed the progression of HCC in mice. This effect may be associated with a direct anti-tumour effect, and/or enhancement of a systemic immune response
Eur J Cancer. 2004 Jun;40(9):1397-403

Nagler RM, Nagler A.
The molecular basis of salivary gland involvement in graft--vs.--host disease.
During the past two decades, the involvement of salivary glands in graft vs. host disease (GVHD) had been intensively researched and published. GVHD occurs in 40-70% of patients treated with bone marrow and peripheral blood stem cell transplantation (PBSCT), and improved survival rates have led to a continuously increasing number of GVHD patients suffering from induced salivary insult. Limited studies suggest that a large percentage of GVHD patients is affected and that the induced salivary dysfunction occurs rapidly following the transplantation. It affects both major and minor salivary glands and reflects the severity of the disease. Moreover, profound sialochemical alterations may be diagnostic of GVHD. An additional reason for this vast research is that GVHD, as an autoimmune-like disease, seemed to be an appropriate model for studying a much more prevalent and well-known and well-studied autoimmune disease involving salivary glands: Sjogren's syndrome. The purpose of the current review-which is, to the best of our knowledge, the first of its kind-is to describe the GVHD-related sialometric and sialochemical data published in the past two decades for both major and minor salivary glands and

to discuss the pathogenesis and molecular basis of the disease.
J Dent Res. 2004 Feb;83(2):98-103

O'Brien ME, Wigler N, Inbar M, Rosso R, Grischke E, Santoro A, Catane R, Kieback DG, Tomczak P, Ackland SP, Orlandi F, Mellars L, Alland L, Tendler C.

CAELYX Breast Cancer Study Group.Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer.

BACKGROUND: This study was designed to demonstrate that efficacy [progression-free survival (PFS)] of CAELYX [pegylated liposomal doxorubicin HCl (PLD)] is non-inferior to doxorubicin with significantly less cardiotoxicity in first-line treatment of women with metastatic breast cancer (MBC). PATIENTS AND METHODS: Women (n=509) with MBC and normal cardiac function were randomized to receive either PLD 50 mg/m2 (every 4 weeks) or doxorubicin 60 mg/m2 (every 3 weeks). Cardiac event rates were based on reductions in left ventricular ejection fraction as a function of cumulative anthracycline dose. RESULTS: PLD and doxorubicin were comparable with respect to PFS [6.9 versus 7.8 months, respectively; hazard ratio (HR)=1.00; 95% confidence interval (CI) 0.82-1.22]. Subgroup results were consistent. Overall risk of cardiotoxicity was significantly higher with doxorubicin than PLD (HR=3.16; 95%CI 1.58-6.31; P<0.001). Overall survival was similar (21 and 22 months for PLD and doxorubicin, respectively; HR=0.94; 95%CI 0.74-1.19). Alopecia (overall, 66% versus 20%; pronounced, 54% versus 7%), nausea (53% versus 37%), vomiting (31% versus 19%) and neutropenia (10% versus 4%) were more often associated with doxorubicin than PLD. Palmar-plantar erythrodysesthesia (48% versus 2%), stomatitis (22% versus 15%) and mucositis (23% versus 13%) were more often associated with PLD than doxorubicin. CONCLUSIONS: In first-line therapy for MBC, PLD provides comparable efficacy to doxorubicin, with significantly reduced cardiotoxicity, myelosuppression, vomiting and alopecia
Ann Oncol. 2004 Mar;15(3):440-9

Ostrovsky O, Nagler A, Korostishevsky M, Gazit E, Galski H.

Genotype and allele frequencies of C3435T polymorphism of the MDR1 gene in various Jewish populations of Israel.

The human multidrug-resistant gene (MDR1) encodes for P-glycoprotein (P-gp), which is a membrane-bound efflux-transporter conferring resistance to a number of natural cytotoxic drugs and potentially toxic xenobiotics. The wobble C3435T polymorphism at exon 26 was associated with different expression levels of the MDR1 gene and substrate uptake. Differences in allele frequencies of the C3435T polymorphism have previously been demonstrated between racial groups. In this study, 500 individuals from 5 Jewish populations of Israel (Ashkenazi, Yemenite, North African, Mediterranean, Near-Eastern) were examined for C3435T polymorphism using a PCR-RFLP-based technique to calculate genotype and allele frequencies. Frequencies of the C allele were quite similar among the Ashkenazi

(0.65), Yemenite (0.645), and North-African (0.615) Jewish populations. However, the frequency of this allele was slightly lower among Mediterranean Jews (0.58) and significantly lower among Near-Eastern Jews (0.445). The frequency of the C allele among Near-Eastern Jews is, therefore, significantly different from those of all other tested Jewish populations. In comparison to previously studied non-Jewish populations, the frequency of this allele among Near-Eastern Jews is different from that in West Africans (0.91) but is similar to that in whites (0.497). However, the C allele frequencies among the other 4 Jewish populations are significantly lower than that found among West Africans and significantly higher than among non-Jewish whites. These data may have important therapeutic and prognostic implication for P-gp-related drug dosage recommendation in Jewish populations.
Ther Drug Monit. 2004 Dec;26(6):679-84.

Paltiel O, Marzec-Boguslawska A, Soskolne V, Massalha S, Avitzour M, Pfeffer R, Cherny N, Peretz T.

Use of tranquilizers and sleeping pills among cancer patients is associated with a poorer quality of life.

PURPOSE: To evaluate the association between sleeping pill/tranquilizer (SP/T) use and quality of life (QOL) among cancer patients. PATIENTS AND METHODS: Oncology patients (n = 909) in three Israeli hospitals were interviewed in clinics, day centers and in-patient departments regarding SP/T use in the previous week. Crude and adjusted QOL scores, measured using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-C30 (EORTC QLQ-C30), were compared in users vs. non-users. RESULTS: Sleeping pill/tranquilizer use was self-reported by 234 (25.7%) participants, but rarely documented in medical charts. Factors associated with SP/T use were female gender (adjusted Odds ratio, OR: 1.79; 95% Confidence interval, CI: 1.22-2.62, age (OR: 4.6; 95% CI: 1.66-12.53 for age 70+), place of birth (OR: 1.97; 95% CI: 1.19-3.26 for Eastern Europe compared with Israel), concomitant use of painkillers (OR: 2.88; 95% CI: 1.97-4.20) and presence of cardiovascular disease (OR: 2.41; 95% CI: 1.48-3.91). Controlling these factors as well as disease status, users had a poorer QOL on all functional scales ($p < 0.001$) as well as global QOL. Furthermore, users reported increased severity of symptoms, especially fatigue, insomnia, pain, dyspnea and constipation ($p < 0.01$), compared to non-users. CONCLUSIONS: Use of SP/T, reported by one fourth of cancer patients, was associated with substantially poorer QOL and increased severity of symptoms. Causal inference is not possible given the cross-sectional design. Periodic inquiry regarding use of these medications in the Oncology Clinic is recommended since it may identify patients with poor QOL and unmet needs.
Qual Life Res. 2004 Dec;13(10):1699-706.

Peled T, Landau E, Mandel J, Glukhman E, Goudsmid NR, Nagler A, Fibach E.

Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice.

OBJECTIVE: We previously demonstrated that cellular copper is involved in the regulation of proliferation and differentiation of hematopoietic progenitor cells.

Modulation of cellular copper was achieved by supplementing the culture with a copper chelator that reduces cell copper content, or copper salts, which elevate the level of cellular copper. In the present study, we evaluated the effect of short-term (3-week) treatment with the copper chelator tetraethylenepentamine (TEPA) on short- and long-term (up to 11 weeks) ex vivo expansion of hematopoietic progenitors, as well as on their SCID engraftment potential. MATERIALS AND METHODS: Cord blood-derived purified CD34+ cells were grown in liquid medium supplemented with the cytokines stem cell factor, thrombopoietin, Flt3 ligand, and IL-6, and the chelator TEPA for the first 3 weeks and then for up to 11 weeks with cytokines alone. Control cultures were supplemented with cytokines alone for the entire culture duration. Cultured cells were characterized by immunophenotyping and cloning (CFUc). Transplantability was assayed by injection of repurified CD34+ cells into NOD/SCID mice. RESULTS: In the short term, TEPA supported increased percentages of early progenitors over control cultures incubated with cytokines alone (CD34(+)CD38-, p=0.001 and CD34(+)Lin-, p=0.016). In the long term, TEPA pretreated cultures showed prolonged expansion of CD34+ cells (p=0.01) and CFUc (p=0.002) compared with that of untreated cultures. The SCID engraftment potential of CD34+ cells repurified from the TEPA-treated cultures was higher compared with that of the control, i.e., only cytokine-treated cultures (p=0.03). CONCLUSION: TEPA enabled preferential proliferation of early progenitor cells with the phenotype CD34(+)CD38- and CD34(+)CD38- Lin- during the first weeks of culture, resulting in the observed increased long-term ex vivo expansion and engraftment capabilities.
Exp Hematol. 2004 Jun;32(6):547-55

Pfeffer MR, Kundel Y, Zehavi M, Catane R, Koller M, Zmora O, Elkayam R, Symon Z

A phase I study of oral UFT given concomitantly with standard preoperative radiotherapy for rectal cancer.

BACKGROUND: Preoperative radiotherapy is standard treatment for rectal cancer and is often combined with 5-fluorouracil-based chemotherapy. UFT, a new oral 5FU derivative, given daily during a course of radiotherapy mimics the effect of continuous-infusion 5FU. OBJECTIVES: To determine the maximum tolerated dose of oral UFT and leucovorin with preoperative pelvic irradiation for rectal cancer, and assess tumor response. METHODS: In this phase 1 trial, 16 patients aged 42-79 years with tumors within 12 cm of the anal verge received radiotherapy, 45 Gy over 5 weeks, an escalating dose of oral UFT, and a fixed dose of 30 mg/day leucovorin. UFT and leucovorin were given for 28 consecutive days concomitant with the first 4 weeks of radiotherapy. Surgery was scheduled for 4-6 weeks after completion of radiotherapy. The surgical procedure was determined by the surgeon at the time of surgery. RESULTS: No grade III toxicity was seen at 200 mg/m2/day UFT. Of eight patients who received 240 mg/m2/day UFT, one developed grade IV diarrhea; of four patients who received 270 mg/m2/day UFT, one was hospitalized with grade IV diarrhea and leukopenic fever and died during hospitalization. Of the 15 evaluable patients, 9 had pathologic tumor downstaging including 4 patients with complete response. Only one patient required a colostomy. CONCLUSIONS: The MTD of UFT together with leucovorin and preoperative radiotherapy for rectal cancer is 240 mg/m2. The major toxicity was diarrhea. Downstaging was noted in 60% of patients, allowing sphincter-preserving surgery even in patients with low tumors
Isr Med Assoc J. 2004 Oct;6(10):595-8

Pfeffer MR, Rabin T, Tsvang L, Goffman J, Rosen N, Symon Z.
Orbital lymphoma: is it necessary to treat the entire orbit?
PURPOSE: Conformal radiotherapy (RT) has been used for all patients with orbital lymphoma treated at our institution since 1997. We retrospectively reviewed the charts of 23 consecutive patients to test the hypothesis that partial orbit RT is effective and less toxic than whole orbit RT. METHODS AND MATERIALS: Twelve patients with limited lesions were treated to partial orbital volumes and 11 patients (1 with bilateral disease) with more extensive lesions received whole orbit RT. The dose was 20-30 Gy (median, 25.2 Gy) for 19 patients with low-grade lymphoma and 24-40 Gy (median, 39.6 Gy) for 5 patients with intermediate- to high-grade lymphoma. The follow-up was 12-68 months (median, 34 months). RESULTS: All patients had a complete response to RT. Intraorbital recurrence developed in previously uninvolved areas not included in the initial target volume in 4 patients (33%) treated with partial orbit RT. All were salvaged by repeat RT or surgery. No patient treated with whole orbit RT developed intraorbital recurrence. The acute and long-term toxicity was similar in both groups. All but 1 patient retained good vision. CONCLUSION: Patients with orbital lymphoma should be treated to the entire orbit. An effective dose of RT for low-grade lesions is 25 Gy, which results in minimal morbidity even when delivered to the entire orbit.
Int J Radiat Oncol Biol Phys. 2004 Oct 1;60(2):527-30

Pinthus JH, Fridman E, Dekel B, Goldberg I, Kaufman-Francis K, Eshhar Z, Harmelin A, Rechavi G, Mor O, Ramon J, Mor Y.
ErbB2 is a tumor associated antigen and a suitable therapeutic target in Wilms tumor.
PURPOSE: The modern multimodality therapeutic approach to Wilms tumor (WT), combining surgery with radiotherapy and chemotherapy results in high cure rates even for high stage disease. However, the combination of radiotherapy and chemotherapy is associated with severe early and late complications such as neutropenic sepsis, growth retardation and secondary malignancies. Therefore, novel therapeutic strategies, which would decrease the treatment burden, are required. We studied the expression of erbB2 growth factor receptor in WT cells as well as its role as a tumor therapeutic target in an in vivo xenograft model of Wilms tumor. MATERIALS AND METHODS: Paraffin embedded pathological samples from 14 different WT cases as well as xenografts derived thereof were immunostained with anti-erbB2 monoclonal antibody. The immunostaining was graded in comparison to a known positive control (breast cancer) and was scored by the intensity of staining (0 to +3) multiplied by the percentage of cells expressing the antigen. The expression of erbB2 in the human WT xenograft was verified also by fluorescence activated cell sorter analysis. In addition, nude mice bearing established subcutaneous human WT xenografts were treated with either 3 intraperitoneal injections of N29 anti-erbB2 monoclonal antibody or irrelevant antibody. RESULTS: All of the authentic human pathological samples, except 1 anaplastic WT as well the WT xenografts (at different stages), expressed erbB2. Expression was also observed in WT metastasis and in tumors which out grew chemotherapy. Systemic administration of anti-erbB2 monoclonal antibody inhibited and even prevented the growth of WT xenograft in vivo. CONCLUSIONS: ErbB2 is a tumor associated antigen in WT. Being expressed in almost all tumor stages, our in vivo model suggests that erbB2 may serve as a WT therapeutic target. Further work is needed to establish the role of erbB2 in the disease

and its potential use in decreasing current treatment burden.
J Urol. 2004 Oct;172(4 Pt 2):1644-8.

Pinthus JH, Waks T, Malina V, Kaufman-Francis K, Harmelin A, Aizenberg I, Kanety H, Ramon J, Eshhar Z.
Adoptive immunotherapy of prostate cancer bone lesions using redirected effector lymphocytes.
Prostate cancer is currently the most commonly diagnosed noncutaneous malignancy in American men. When metastatic, usually to the bone, the disease is no longer curable and is usually treated palliatively with androgen ablation. However, after conversion to androgen-independent disease, there is no effective therapy currently available. The "T body" approach, which uses genetically reprogrammed lymphocytes derived from the patient and expressing chimeric receptor genes, combines the effector functions of T lymphocytes and NK cells with the ability of antibodies to recognize predefined surface antigens with high specificity and in a non-MHC-restricted manner. We show here the therapeutic efficacy of human lymphocytes bearing erbB2-specific chimeric receptors on human prostate cancer BM lesions in a SCID mouse model after conditioning of the recipient to allow homing and persistent functioning of the adoptively transferred cells. Induction of stromal cell-derived factor-1 production within the BM using low-dose irradiation or cyclophosphamide combined with IL-2 administration enhanced the homing of systemically delivered T bodies, resulting in decreased tumor growth and prostate-specific antigen secretion, prolongation of survival, and even cure of the treated mice. These preclinical studies strongly support the idea that the T body approach has therapeutic potential in disseminated prostate cancer
J Clin Invest.2004 Dec;114(12):1774-81

Raanani P, Ben-Bassat I, Gan S, Trakhtenbrot L, Mark Z, Ashur-Fabian O, Itskovich S, Brok-Simoni F, Rechavi G, Amariglio N, Nagler A.
Assessment of the response to imatinib in chronic myeloid leukemia patients--comparison between the FISH, multiplex and RT-PCR methods.
OBJECTIVE: The objective of this study was to evaluate the kinetics of molecular response in chronic myeloid leukemia (CML) patients treated with imatinib and to compare between the fluorescent in situ hybridization (FISH), multiplex and real-time quantitative RT-PCR (RQ-PCR) methods with this respect. METHODS: Molecular follow-up was carried out on 24 CML patients treated with imatinib. FISH analysis was performed according to the standard protocol. For RT-PCR the multiplex and RQ-PCR methods were used. RESULTS: Sixty-three percent and 52% of the patients achieved complete remission according to FISH and multiplex RT-PCR analyses, respectively. Seventy-five percent of the patients achieved remission within the first year of treatment. In 83% of the cases the FISH and RT-PCR results were concordant. RQ-PCR analysis was carried out on 32 of the 41 samples negative by multiplex RT-PCR but only nine were negative. All samples with a BCR-ABL/ABL ratio below 2% were also negative by FISH. There was an excellent correlation between the RQ-PCR and the FISH tests. CONCLUSIONS: Molecular remission according to FISH and multiplex RT-PCR can be achieved by imatinib within 1 yr of therapy. There is a good correlation between the FISH, multiplex and RQ-PCR results in terms of the kinetics of disappearance of the BCR-ABL transcript and the predictability of each method for the other. Although RQ-PCR is the most sensitive method for molecular follow-up, FISH and multiplex RT-PCR can be used as complementary tools, at least

during the early period of treatment
Eur J Haematol. 2004 Oct;73(4):243-50

Raanani P, Ben-Bassat I.
Detection of minimal residual disease in acute myelogenous leukemia.
Acute myelogenous leukemia (AML) is considered to be in complete remission when fewer than 5% of the cells in bone marrow are blasts. Nevertheless, approximately two thirds of patients relapse due to persisting leukemic blasts. The persistence of these cells, below the threshold of morphological detection, is termed minimal residual disease (MRD) and various methods are used for its detection. These methods include classical cytogenetics, fluorescence in situ hybridization, qualitative and quantitative RT-PCR and multiparametric flow cytometry. Currently, less than half of the AML patients have a specific marker detectable by RT-PCR techniques. The major specific molecular markers are involvement of the MLL gene with up to 50 different partners and partial tandem duplications, the core binding factor leukemias with AML1/ETO and CBFbeta/MYH11 rearrangements, PML/RARalpha in acute promyelocytic leukemia, internal tandem duplications and mutations of FLT3 and some other rare translocations. In addition, several other genes show abnormal expression levels in AML, including the Wilms tumor gene, the PRAME gene and Ig/TCR rearrangements. Most of these genetic abnormalities can be detected by qualitative but more importantly by quantitative RT-PCR. The kinetics of disappearance of molecular markers in AML differs between the various types of leukemias, although at least a 2 log reduction of transcript after induction chemotherapy is necessary for long-term remission in all types. Conversely, the change of PCR from negativity to positivity is highly predictive of relapse. Whereas in acute lymphoblastic leukemia, multiparametric flow cytometry is an established method for MRD detection, this is less so in AML. The reason is the absence of well-characterized leukemia-specific antigens and the existence of phenotypic changes at relapse. On the other hand, this method is convenient due to its simplicity and universal applicability. In conclusion, several methods can be used for MRD detection in AML patients; each has its pros and cons. Several issues still remain to be settled including the choice of the best method and the timing for MRD monitoring and above all the practical clinical implications of MRD in the various types of AML.
Acta Haematol. 2004;112(1-2):40-54

Rosenberg N, Landau M, Luboshitz J, Rechavi G, Seligsohn U.
A novel Phe171Cys mutation in integrin alpha causes Glanzmann thrombasthenia by abrogating alphabeta complex formation.
BACKGROUND: Glanzmann thrombasthenia (GT) is an autosomal recessive bleeding disorder characterized by lack of platelet aggregation induced by most agonists. The disease is caused by mutations in either alpha(IIb)[glycoprotein (GP) IIb] or beta(3) (GPIIIa) genes that lead to a lack or dysfunction of the integrin alpha(IIb)beta(3) which serves as a fibrinogen receptor. PATIENTS: Mucocutaneous bleeding manifestations and platelet dysfunction consistent with GT were observed in three members of a Cypriot family: a 3-year-old proband, her father and her paternal uncle. OBJECTIVE: To determine the molecular basis of GT in this family and to characterize possible biochemical and structural defects. RESULTS: Analysis of the patients' platelets by fluorescence-activated cell sorting demonstrated trace amounts of beta(3), no alpha(IIb) and no alpha(IIb)beta(3) on the membrane. Sequence analysis revealed a novel T607G transversion in exon 5 of the alpha(IIb) gene

predicting a Phe171Cys alteration that created a PstI recognition site. All three patients were homozygous for the mutation, the mother and paternal grandparents of the proband were heterozygous, whereas 110 healthy subjects lacked this transversion. Chinese hamster ovary cells cotransfected with cDNAs of mutated alpha(IIb) and wild-type beta(3) failed to express alpha(IIb)beta(3) as shown by immunoprecipitation and immunohistochemistry experiments. Structural analysis of the alpha(IIb)beta(3) model, which was based on the crystal structure of alpha(v)beta(3), indicated that Phe171 plays an essential role in the interface between the beta-propeller domain of alpha(IIb) and the betaA domain of beta(3). CONCLUSIONS: A novel Phe171Cys mutation in the alpha(IIb) gene of patients with GT is associated with abrogation of alpha(IIb)beta(3) complex formation
J Thromb Haemost. 2004 Jul;2(7):1167-75.

Roth Y, Tichler T, Kostenich G, Ruiz-Cabello J, Maier SE, Cohen JS, Orenstein A, Mardor Y.
High-b-value diffusion-weighted MR imaging for pretreatment prediction and early monitoring of tumor response to therapy in mice.
PURPOSE: To evaluate the use of diffusion-weighted magnetic resonance (MR) imaging with standard and high b values for pretreatment prediction and early detection of tumor response to various antineoplastic therapies in an animal model. MATERIALS AND METHODS: Mice bearing C26 colon carcinoma tumors were treated with doxorubicin (n = 25) and with aminolevulinic acid-based photodynamic therapy (n = 23). Fourteen mice served as controls. Conventional T2-weighted fast spin-echo and diffusion-weighted MR images were acquired once before therapy and at 6, 24, and 48 hours after treatment. Pretreatment and early (1-2 days) posttreatment water diffusion parameters were calculated and compared with later changes in tumor volumes measured on conventional MR images by using the Pearson correlation test. RESULTS: In chemotherapy-treated tumors, a significant correlation (P <.002, r = 0.6) was observed between diffusion parameters that reflected tumor viability, measured prior to treatment, and changes in tumor volumes after therapy. This correlation implies that tumors with high pretreatment viability will respond better to chemotherapy than more necrotic tumors. In tumors treated with photodynamic therapy, no such correlation was found. Changes observed in water diffusion 1-2 days after treatment significantly correlated with later tumor growth rate for both therapies (P <.002, r = 0.54 for photodynamic therapy; P <.0003, r = 0.61 for chemotherapy). CONCLUSION: High-b-value diffusion-weighted MR imaging has potential use for the early detection of response to therapy and for predicting treatment outcome prior to initiation of chemotherapy.
Radiology. 2004 Sep;232(3):685-92.

Sadetzki S, Calderon-Margalit R, Modan B, Srivastava S, Tuttle RM.
Ret/PTC activation in benign and malignant thyroid tumors arising in a population exposed to low-dose external-beam irradiation in childhood.
Ionizing radiation is the strongest risk factor known for the development of thyroid neoplasia. Although ret/PTC rearrangements have been identified in both spontaneous and radiation-induced papillary thyroid cancer, they seem more frequent among radiation-associated tumors. We studied the frequency of ret/PTC activation in a group of sporadic and radiation-induced thyroid carcinomas (n = 49) and adenomas (n = 13) among 44 individuals treated for Tinea Capitis with low-dose external irradiation as well as in 18 nonirradiated subjects. Total RNA recovered from

paraffin-embedded thyroid cancer surgical specimens was analyzed for ret/PTC 1, 2, and 3 mutations using RT-PCR with Southern blotting to maximize detection sensitivity. Ret/PTC rearrangements were identified in 42.9% of thyroid carcinoma and 46.2% of adenoma subjects. Among the positive carcinoma specimens, three were follicular carcinomas. Ret/PTC 1, the predominant rearrangement, was more prevalent in nonirradiated compared with irradiated carcinomas (66.7 vs. 27.0%; P = 0.04). Ret/PTC activation was associated with male gender. The strengths of this study included analysis of age-, gender-, and ethnicity-matched groups; molecular analysis using two techniques; and a complete blinding of laboratory analysis from clinical features. The differences seen between these and other published results may be related to differences in radiation doses to the thyroid, latency period between time of radiation exposure and development of clinically apparent thyroid cancer, and ethnic background of the study populations.
J Clin Endocrinol Metab. 2004 May;89(5):2281-9

Samira S, Ferrand C, Peled A, Nagler A, Tovbin Y, Ben-Hur H, Taylor N, Globerson A, Lapidot T.
Tumor necrosis factor promotes human T-cell development in nonobese diabetic/severe combined immunodeficient mice.
A major problem after clinical hematopoietic stem cell transplantations is poor T-cell reconstitution. Studying the mechanisms underlying this concern is hampered, because experimental transplantation of human stem and progenitor cells into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice usually results in low T-lymphocyte reconstitution. Because tumor necrosis factor alpha (TNFalpha) has been proposed to play a role in T-lineage commitment and differentiation in vitro, we investigated its potential to augment human T-cell development in vivo. Administration of TNF to irradiated NOD/SCID mice before transplantation of human mononuclear cells from either cord blood or adult G-CSF-mobilized peripheral blood (MPBL) led 2-3 weeks after transplantation to the emergence of human immature CD4(+)CD8(+) double-positive T-cells in the bone marrow (BM), spleen, and thymus, and in this organ, the human cells also express CD1a marker. One to 2 weeks later, single-positive CD4(+) and CD8(+) cells expressing heterogenous T-cell receptor alpha beta were detected in all three organs. These cells were also capable of migrating through the blood circulation. Interestingly, human T-cell development in these mice was associated with a significant reduction in immature lymphoid human CD19(+) B cells and natural killer progenitors in the murine BM. The human T cells were mostly derived from the transplanted immature CD34(+) cells. This study demonstrates the potential of TNF to rapidly augment human T lymphopoiesis in vivo and also provides clinically relevant evidence for this process with adult MPBL progenitors.
Stem Cells. 2004;22(6):1085-100

Shimon I, Hadani M, Nass D, Zwas ST.
Malignant bronchial carcinoid tumor metastatic to the pituitary in a thyroid carcinoma patient: successful treatment with surgery, radiotherapy and somatostatin analog.
A carcinoid pituitary metastasis is very rare, and is reported scarcely in a few patients. We describe an unusual case of metastatic atypical bronchial carcinoid to the anterior pituitary gland in a 47-year-old male who presented with bitemporal hemianopsia and hypopituitarism. His primary bronchial carcinoid was resected two years previously.

Foci of metastatic papillary thyroid carcinoma were also identified in the lung resected for the bronchial carcinoid. He thereby underwent total thyroidectomy followed by radioiodine ablative treatment. Transsphenoidal partial removal of the suprasellar mass was performed, and atypical carcinoid metastasis was identified. He received conventional fractionated sellar radiotherapy, which was supplemented with octreotide (Sandostatin LAR) injections following a positive pituitary uptake on octreotide scan. This treatment suppressed his elevated 5-HIAA urinary excretion to a normal level. His vision has returned to normal and the pituitary mass diminished in size.
Pituitary. 2004;7(1):51-7.

Shimoni A, Nagler A.
Clinical implications of minimal residual disease monitoring for stem cell transplantation after reduced intensity and nonmyeloablative conditioning.
Allogeneic stem cell transplantation (SCT) is a potentially curative therapy for a variety of hematological malignancies; however, relapse and treatment-related toxicities are major obstacles to cure. Nonmyeloablative and reduced-intensity conditioning regimens were designed not to eradicate the malignancy completely, but rather to be immunosuppressive enough to allow engraftment, and to serve as a platform for additional cellular immunotherapy. Minimal residual disease (MRD) typically persists after SCT, and is gradually eliminated with different kinetics typical of each disease. Significant progress has been achieved with technologies for MRD assessment. Quantitative PCR tests are very sensitive in detecting tumor-associated transcripts, allowing serial monitoring. Threshold levels have been established for some malignancies, above which relapse is imminent. Persistent negative tests, a low level or a decreasing MRD level are consistent with continuous remission, whereas high-level MRD or increasing levels predict an incipient relapse. Patients at high risk of relapse are candidates for additional cellular or targeted therapy. Immunotherapy is more effective for MRD than at frank relapse. Timing and dosing of therapy are not yet well established and depend on aggressiveness of the disease, type of conditioning, level and kinetics of MRD.
Acta Haematol. 2004;112(1-2):93-104

Shimoni A, Nagler A.
Nonmyeloablative stem cell transplantation: lessons from the first decade of clinical experience.
Allogeneic stem cell transplantation is an effective and potentially curative therapy for hematologic malignancies. However, the procedure may be associated with significant morbidity and mortality resulting from toxicity of the conditioning regimen, limiting it to younger patients in good medical conditioning. Over the past decade, nonmyeloablative and reduced-intensity conditioning regimens have been designed to reduce toxicity and allow stem cell transplantation in elderly and medically infirm patients. These are relatively nontoxic and tolerable regimens designed not to maximally eradicate the malignancy, but rather to provide sufficient immune suppression to achieve engraftment and to allow induction of graft-versus-leukemia effect as the primary treatment. After transplantation, immune interventions are often required to hasten this graft-versus-leukemia effect. In this review, we discuss emerging data defining the relative toxicities and outcomes after nonmyeloablative transplantation in certain settings and our approach to patient selection and post-transplant immune interventions trying to improve overall

outcome.
Curr Hematol Rep. 2004 Jul;3(4):242-8

Shimoni A, Yeshurun M, Hardan I, Avigdor A, Ben-Bassat I, Nagler A.
Hrombotic microangiopathy after allogeneic stem cell transplantation in the era of reduced-intensity conditioning: The incidence is not reduced.
Thrombotic microangiopathy (TMA) is one of the most severe complications of stem cell transplantation (SCT). Endothelial cell injury caused by the toxic effects of high-dose chemoradiotherapy is likely the primary event in pathogenesis. The incidence, clinical settings, and risk factors for TMA in the era of nonmyeloablative conditioning have not been well defined. The data on 147 consecutive SCTs in a single center were collected, and patients with TMA were identified. Patient characteristics, response to therapy, and outcome were recorded, and risk factors were determined. TMA occurred in 22 of 147 transplantations, with a projected incidence of 20% +/- 4%. TMA occurred in 3 clinical settings: classic multifactorial TMA, TMA associated with severe hepatic graft-versus-host disease (GVHD), and TMA associated with second SCT, with a projected incidence of 8% +/- 3%, 73% +/- 14%, and 70% +/- 16% of patients at risk, respectively. TMA occurred after 23% +/- 6% of nonmyeloablative and 16% +/- 5% of myeloablative conditioning regimens (not significant). Univariate analysis determined SCT from unrelated donors, SCT during advanced or active disease, second SCT within 6 months of a prior SCT, and acute GVHD as risk factors for TMA. The last 2 factors remained significant in a multivariate model. Thirty-two percent of patients responded to therapy. The peri-TMA mortality rate was 68% +/- 10%. Six patients had diffuse alveolar hemorrhage complicating TMA. SCT-associated TMA is a relatively common complication with unsatisfactory therapy and grim prognosis. Fludarabine-based nonmyeloablative conditioning does not confer a lesser risk for TMA. This observation may relate to the selective use of these regimens in elderly and heavily pretreated patients or to the lack of reduction of GVHD with these regimens, and fludarabine itself may be involved in causing endothelial damage. Further exploration of novel preventive and therapeutic measurements is required in high-risk settings.
Biol Blood Marrow Transplant. 2004 Jul;10(7):484-93.

Somech R, Izraeli S, J Simon A.
Histone deacetylase inhibitors--a new tool to treat cancer.
Transcriptional regulation in eukaryotes is a multilevel hierarchical process. It is becoming clear that higher-order chromatin structure, occurring via modifications of histones in their nucleosome structure, plays a crucial role in regulating gene expression, both in normal and pathological states. Deacetylation of histones by histone deacetylases (HDACs) modifies the chromatin from an open gene active euchromatin structure to a closed gene silenced heterochromatin structure. Several cancer promoting mutations and chromosomal translocations result in repression of transcription through abnormal recruitment and activation of HDACs, leading to neoplastic transformation. This is the rationale for the evolvement of HDAC inhibitors as a new class in cancer therapy. Trials have shown anti-proliferation effect of histone deacetylase inhibitors in cell culture, animal models and in human with both hematological and solid tumors. The exact mechanism by which histone deacetylase inhibitors exert their effect is still obscure. Reversal of the alteration in gene expression by fusion transcription factors or overexpressed repressors is just one of several possible explanations. The territory of heterochromatin in the vicinity of the

nuclear periphery raised the possibility of involvement of nuclear envelope proteins in the regulation of transcription. Our laboratory is interested in the transcription repression mechanism induced by the nuclear envelope lamina associated polypeptide 2 (LAP2) family of proteins through chromatin modification. Here, we will describe the structure of the nucleosome, review regulation of gene expression by acetylation of histones and give an update on the current phase I and phase II clinical trials with histone deacetylase inhibitors.
Cancer Treat Rev. 2004 Aug;30(5):461-72

Spiegel A, Kollet O, Peled A, Abel L, Nagler A, Bielorai B, Rechavi G, Vormoor J, Lapidot T.
Unique SDF-1-induced activation of human precursor-B ALL cells as a result of altered CXCR4 expression and signaling.
The mechanisms governing migration and extramedullary dissemination of leukemic cells remain obscure. In this study the migration and in vivo homing to the bone marrow of nonobese diabetic severe combined immunodeficient (NOD/SCID) mice injected with human precursor-B acute lymphoblastic leukemia (ALL) cells in comparison to normal CD34+ progenitors (both cord blood and mobilized peripheral blood) was investigated. Although migration and homing of both cell populations was dependent on stromal cell-derived factor 1 (SDF-1)/CXCR4 interactions, major differences in receptor expression as well as the migratory capacity toward various concentrations of SDF-1 were found. Furthermore, unlike normal CD34+ progenitors, in vivo homing of the leukemic cells was superior when recipient NOD/SCID mice were not irradiated prior to transplantation. In addition, we report differences in the adhesion molecules activated following SDF-1 stimulation, documenting a major role for very late antigen 4 (VLA-4), but not VLA-5 and lymphocyte function-associated antigen-1 (LFA-1), in homing of precursor-B ALL cells. Interestingly, Toxin-B and pertussis toxin inhibited the homing of the leukemic cells but not that of normal CD34+ progenitors or normal CD10+/CD19+ precursor-B cells, revealing differences in CXCR4 signaling pathways that are based on changes that acquired by the leukemic cells. Altogether, our data provide new insights into different SDF-1-induced signaling, activation, and consequent motility between normal CD34+ and precursor-B ALL progenitors, which may lead to improved clinical protocols.
Blood. 2004 Apr 15;103(8):2900-7

Sredni B, Weil M, Khomenok G, Lebenthal I, Teitz S, Mardor Y, Ram Z, Orenstein A, Kershenovich A, Michowiz S, Cohen YI, Rappaport ZH, Freidkin I, Albeck M, Longo DL, Kalechman Y.
Ammonium trichloro(dioxoethylene-o,o')tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop.
Cancer cells of different solid and hematopoietic tumors express growth factors in respective stages of tumor progression, which by autocrine and paracrine effects enable them to grow autonomously. Here we show that the murine B16 melanoma cell line and two human primary cultures of stomach adenocarcinoma and glioblastoma multiforme (GBM) constitutively secrete interleukin (IL)-10 in an autocrine/paracrine manner. This cytokine is essential for tumor cell proliferation because its neutralization decreases clonogenicity of malignant cells, whereas addition of recombinant IL-10 increases cell proliferation. The immunomodulator ammonium

trichloro(dioxoethylene-o,o')tellurate (AS101) decreased cell proliferation by inhibiting IL-10. This activity was abrogated by exogenous addition of recombinant IL-10. IL-10 inhibition by AS101 results in dephosphorylation of Stat3, followed by reduced expression of Bcl-2. Moreover, these activities of AS101 are associated with sensitization of tumor cells to chemotherapeutic drugs, resulting in their increased apoptosis. More importantly, AS101 sensitizes the human aggressive GBM tumor to paclitaxel both in vitro and in vivo by virtue of IL-10 inhibition. AS101 sensitizes GBM cells to paclitaxel at concentrations that do not affect tumor cells. This sensitization can also be obtained by transfection of GBM cells with IL-10 antisense oligonucleotides. Sensitization of GBM tumors to paclitaxel (Taxol) in vivo was obtained by either AS101 or by implantation of antisense IL-10-transfected cells. The results indicate that the IL-10 autocrine/paracrine loop plays an important role in the resistance of certain tumors to chemotherapeutic drugs. Therefore, anti-IL-10 treatment modalities with compounds such as AS101, combined with chemotherapy, may be effective in the treatment of certain malignancies.
Cancer Res. 2004 Mar 1;64(5):1843-52

Stahl S, Bar-Meir E, Friedman E, Regev E, Orenstein A, Winkler E.
Genetics in melanoma.
Melanoma is the leading cause of death from skin tumors worldwide, with an annual increase in incidence over the past decade. The molecular mechanisms involved in melanoma pathogenesis are beginning to be unraveled. While a family history of melanoma and exposure to ultraviolet irradiation have been known for years as risk factors in melanoma development, the precise genes involved in inherited predisposition were defined only in the past decade. Germline mutations in two genes that play a pivotal role in controlling cell cycle and division--CDKN2A and cyclin-dependent kinase 4 (CDK4)--have been detected in autosomal, dominant, high penetrance familial melanoma cases. In addition to these two highly penetrant genes, germline mutations and polymorphisms in a few low penetrance genes have been reported in familial melanoma cases: melanocortin-1 receptor, epidermal growth factor, glutathione s-transferase M1, cytochrome p450 debrisoquine hydroxylase locus (CYP2D6) and vitamin D receptor.
Isr Med Assoc J. 2004 Dec;6(12):774-7

Starinsky S, Figer A, Ben-Asher E, Geva R, Flex D, Fidder HH, Zidan J, Lancet D, Friedman E.
Genotype phenotype correlations in Israeli colorectal cancer patients.
While genetic factors clearly play a key role in colorectal cancer (CRC) pathogenesis and in determining its phenotypic features, the precise genes that involved are largely unknown. To gain insight into these genes, consecutive Israeli CRC patients were genotyped using SNPs from within candidate genes: APC, beta-Catenin, K-RAS, DCC, P16, PTEN, RB1, P15, APOE, ERCC2, P53, MTHFR and hMSH2. Genotyping of consecutive, unselected colorectal cancer patients was done mostly by utilizing the MassARRAY technology (Sequenom) and to a lesser extent DGGE, ARMS and direct DNA sequencing. Correlation of genotypes with specific phenotypic features was carried out for all patients and separately for the Ashkenazim. Overall, 456 patients were analyzed, the majority (64.25%) being of Ashkenazi origin; mean age at diagnosis was 65.6 +/- 14 (range 25-90 years), and the mean follow-up was 4.7 +/- 0.28 (range 0-30 years). Statistically significant associations were noted between SNPs in beta-catenin and APOE and a positive family history of cancer (beta-catenin:

p=0.034, APOE: p=0.033); tumor location and a DCC SNP (p=0.038) and the P53 R72P mutation and survival (p=0.0336). In Ashkenazi patients, ERCC2 and MTHFR genes' SNPs were associated with age at diagnosis (ERCC2: p=0.025, MTHFR: p=0.0005); a P53 polymorphism, APOE and Rb SNPs with a family history of cancer (P53 p=0.034;APOE p=0.04, Rb p= 0.022); DCC SNP with tumor location (p=0.014); and p15 SNP with tumor grade (p=0.032). This preliminary study shows that genetic factors play a role in determining CRC phenotypic features and that a larger cohort with longer follow-up is clearly needed.
Int J Cancer. 2004 Nov 2;114(1):58-73

Tabori U, Beni-Adani L, Dvir R, Burstein Y, Feldman Z, Pessach I, Rechavi G, Constantini S, Toren A.

Risk of venous thromboembolism in pediatric patients with brain tumors.
BACKGROUND: Venous thromboembolism (VTE) is a common event in adults with malignant brain tumors approaching 24% throughout the course of the disease. The high morbidity and mortality of this complication yielded several protocols for prevention of the disease in adults undergoing neurosurgery for brain tumors and possible primary prevention afterwards. We investigated the incidence and complications of VTE in pediatric neuro-oncology patients. PROCEDURE: We analyzed, retrospectively, the files of all consecutive patients under the age of 18 years who were hospitalized for the treatment of brain tumors between the years 1990 and 2003 in two leading, closely related, Israeli neuro-oncology centers. RESULTS: A total of 462 children were analyzed. Three hundred eighty-four patients underwent surgery and 78 were treated medically. Only three (0.64%) of the patients developed clinical episodes of VTE that were treated conservatively. Two of these patients developed intracranial bleeding while on secondary prevention for the disease. CONCLUSIONS: Although this study has considerable limitations in terms of retrospective design, heterogeneous group of patients and diagnoses, the changing awareness for thrombosis over the last 14 years and the inclusion of symptomatic VTE events only, our surprising data suggest that, as opposed to adults, the risk of clinically significant VTE in children with brain tumors may be exceedingly low. These findings set the stage for future forthcoming evaluations in view of the prospective studies that were done in adults and the possible significant implications for the prevention and possible etiologies of the disease.
Pediatr Blood Cancer. 2004 Nov;43(6):633-6.

Tabori U, Mark Z, Amariglio N, Etzioni A, Golan H, Biloray B, Toren A, Rechavi G, Dalal I.

Detection of RAG mutations and prenatal diagnosis in families presenting with either T-B- severe combined immunodeficiency or Omenn's syndrome.
It has been recently shown that mutations in both of the recombination activating genes RAG1 and RAG2 are involved in each of the two different types of severe combined immunodeficiency (SCID) syndromes: T-B- SCID and Omenn's syndrome (OS). The objective of the study was to search for novel mutations in the RAG genes and to offer prenatal diagnosis for families that have been identified as at risk of T-B- SCID or OS. Mutation analyses of polymerase chain reaction products of RAG1/RAG2 genes were performed in 14 cases (T-B- SCID = 6 and OS = 8). Consanguinity was reported in seven (50%) families. Four missense mutations in the RAG2 gene in six of eight OS patients and in four of six T-B- SCID patients were

detected. The C1845T transition leading to a Tre215Ile substitution is a novel mutation. All but one of the patients were homozygous for the detected mutations, possibly reflecting the consanguinity in these families and the relative rarity of the disease-causing mutations. In addition, three putative polymorphic sites were found. Prenatal diagnosis was offered to seven families, but three of them declined genetic counseling for religious reasons. In the remaining families, four pregnancies were successfully completed, and in one case, the family chose to have an abortion because of a homozygous mutation. Mutations in RAG1/RAG2 genes were detected in only some of the T-B- SCID or OS patients, and the molecular basis for the remaining cases has yet to be elucidated. Important factors such as religious beliefs need to be considered when offering prenatal diagnosis to certain families.
Clin Genet. 2004 Apr;65(4):322-6.

Tal G, Mandelberg A, Dalal I, Cesar K, Somekh E, Tal A, Oron A, Itskovich S, Ballin A, Houri S, Beigelman A, Lider O, Rechavi G, Amariglio N.
Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease.
BACKGROUND: The clinical spectrum of respiratory syncytial virus (RSV) bronchiolitis in previously healthy infants is extremely variable. Thus, it is likely that factors such as genetic heterogeneity contribute to disease severity. Toll-like receptor 4 (TLR4) and CD14 are part of a receptor complex involved in the innate immune response to RSV. METHODS: The association of the TLR4 mutations (Asp299Gly and Thr399Ile) and the CD14/-159 polymorphism were analyzed in 99 infants hospitalized with severe RSV bronchiolitis (group I). Eighty-two ambulatory infants with mild RSV bronchiolitis (group II) and 90 healthy adults (group III) composed the 2 control groups. The TLR4 mutations and the CD14/-159 polymorphism were genotyped by use of reverse-transcriptase polymerase chain reaction and restriction fragment-length polymorphism analysis, respectively. RESULTS: Each of the TLR4 mutations, either alone or in cosegregation, were associated with severe RSV bronchiolitis: the Asp299Gly and Thr399Ile mutations were significantly overrepresented in group I, compared with groups II and III. No association between the CD14/-159 polymorphism and RSV bronchiolitis was found. CONCLUSIONS: These findings suggest that TLR4 mutations, but not the CD14/-159 polymorphism, are associated with an increased risk of severe RSV bronchiolitis in previously healthy infants.
J Infect Dis. 2004 Jun 1;189(11):2057-63.

Trakhtenbrot L, Rechavi G, Amariglio N.
The multiparametric scanning system for evaluation of minimal residual disease in hematological malignancies.
Combined simultaneous analysis of morphology, immunophenotyping and fluorescence in situ hybridization on the same cell offers advantages that may help to disclose the relevance of minimal residual disease (MRD) detection. Morphological analysis of small populations of cells related either to malignancy or recipient-associated markers may improve the accuracy of chimerism and MRD testing and delineate their clinical significance.
Acta Haematol. 2004;112(1-2):24-9

Waldman D, Weintraub M, Freeman A, Neumann Y, Rechavi G, Toren A.
Favorable early response of secondary chronic myeloid leukemia to imatinib.

Second malignant neoplasms are gradually becoming a recognized long-term complication of successful cancer treatment, and they usually respond poorly to conventional therapy and have an unfavorable outcome. Chronic myeloid leukemia (CML) is a clonal panmyelopathy, rarely seen in children with a specific cytogenetic aberration-the Philadelphia chromosome. The translocation generates an aberrant tyrosine kinase, which drives the malignant process in CML and which is also the molecular target for successful treatment of CML with imatinib. It is also exceedingly rare as a secondary malignant neoplasm in both adults and children. We report two cases of secondary CML. The first occurred after successful treatment for nasopharyngeal carcinoma in a child, and the second after treatment for lymphoma in an adolescent. Both patients had an excellent response to treatment with imatinib and attained complete cytogenetic remissions. We conclude that secondary CML may respond favorably to treatment with imatinib.
Am J Hematol. 2004 Apr;75(4):217-9

Weinberg NM, Zwas DR, Owen AN, Zangrilli JG, Van Tassell P.
Left ventricular intracardiac metastatic germ cell tumor presenting with hemorrhagic cerebrovascular event.
We describe an unusual case of a 26-year-old man admitted with respiratory distress and found to have testicular cancer metastatic to the lung and heart. Twelve days after admission, the patient experienced multiple hemorrhagic strokes. Echocardiography demonstrated testicular cancer metastatic to the septal surface of the left ventricle of the heart with presumed embolization to the cerebrovascular region. The patient received chemotherapy and radiation therapy to the areas of tumor mass with subsequent resolution of tumor burden. This is the first reported case of metastasis from embryonal carcinoma of the testis to the left ventricle of the heart.
J Am Soc Echocardiogr. 2004 Oct;17(10):1080-3

Weisz B, Meirow D, Schiff E, Lishner M.
Impact and treatment of cancer during pregnancy.
Cancer is the second most common cause of death in the reproductive years and complicates up to one in 1000 pregnancies. When cancer is diagnosed during pregnancy, the management strategy must take into account both the mother and developing fetus. In this article, the four most common malignancies diagnosed in pregnant patients - cervical and breast cancer, malignant melanoma and lymphoma - will be reviewed, with an emphasis on the impact of the diagnosis and management on the pregnant patient and the developing fetus.
Expert Rev Anticancer Ther. 2004 Oct;4(5):889-902

Zmora O, Dasilva GM, Gurland B, Pfeffer R, Koller M, Nogueras JJ, Wexner SD.
Does rectal wall tumor eradication with preoperative chemoradiation permit a change in the operative strategy?
PURPOSE: Preoperative chemoradiation may downstage locally advanced rectal cancer and, in some cases, with no residual tumor. The management of complete response is controversial and recent data suggest that radical surgery may be avoided in selected cases. Transanal excision of the scar may determine the rectal wall response to chemoradiation. This study was designed to assess whether the absence of tumor in the bowel wall corresponds to the absence of tumor in the mesorectum, known as true complete response. METHODS: A retrospective review of the medical

records of patients who underwent preoperative chemoradiation for advanced mid (6-11 cm from the anal verge) and low (from the dentate line to 5 cm from the anal verge) rectal cancer (uT2-uT3) followed by radical surgery with total mesorectal excision was undertaken. Patients in whom the pathology specimen showed no residual tumor in the rectal wall (yT0, "y" signifies pathologic staging in postradiation patients) were assessed for tumoral involvement of the mesorectum. RESULTS: A total of 109 patients underwent preoperative, high-dose radiation therapy (94 percent with 5-fluorouracil chemosensitization), followed by radical surgery for advanced rectal cancer. Preoperatively, 47 patients were clinically assessed to have potentially complete response. After radical rectal resection, pathology did not reveal any residual tumor within the rectal wall (yT0) in 17 patients. In two (12 percent) of these patients, the mesorectum was found to be positive for malignancy: one had positive lymph nodes that harbored cancer; one had tumor deposits in the mesorectal tissue. CONCLUSIONS: Compete rectal wall tumor eradication does not necessarily imply complete response, because the mesorectum may harbor tumor cells. Thus, caution should be exercised when considering the avoidance of radical surgery. Reliable imaging methods and clinical predictors for favorable outcome are important to allow less radical approaches in the future.

Dis Colon Rectum. 2004 Oct;47(10):1607-12