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R&R's Week in Review: Oncology and Stem Cell Sectors

REVIEW IN ONCOLOGY

During the week of July 12, 2009 (from July 12, 2009 to July 18, 2009) significant news items pertaining to the oncology sector were reported including:

1. Oncolytics Biotech (ONCY, Market Outperform) presented data demonstrating the synergy of reovirus in combination with temozolomide and radiation for the treatment of glioma xenografts at the 28th American Society for Virology Annual Meeting and separately published research on the synergy of reovirus and chemotherapy for the treatment of non-small cell lung cancer (NSCLC);

2. Genta (GETA.OB, Market Perform) reported publication of a paper that independently confirms the link of a key biomarker, a tumor-derived enzyme known as lactate dehydrogenase (LDH), to overall survival in patients with advanced melanoma;

3. CytRx's (CYTR, Market Outperform) investigational cancer drug INNO-206 caused a dramatic destruction of implanted tumors in an experimental animal model of breast cancer, performing considerably better than the broadly used and generally effective chemotherapeutic drug doxorubicin;

4. Array BioPharma (ARRY, Market Outperform) filed an investigational new drug (IND) application with the FDA to initiate a Phase 1 clinical trial evaluating the company's most advanced wholly owned MEK inhibitor, ARRY-162, for the treatment of range of cancers;

5. OSI Pharmaceuticals' (OSIP, Not Rated) and Genentech / Roche's (RO.SW, Not Rated) pivotal Phase 3 study evaluating Tarceva for the treatment of advanced NSCLC, met a key secondary endpoint of extending overall survival in patients who received Tarceva after chemotherapy; and

6. Health Discovery's (HDVY.OB, Not Rated) publication demonstrating the clinical validity of a 4-gene prostate cancer test licensed for commercialization to Abbott (ABT, Not Rated), Quest Diagnostics (DGX, Not Rated), and Clariant (CLRT, Not Rated) has been approved for publication.

Other companies mentioned in this report include:

1. Watson Pharmaceuticals (WPI, Not Rated)
2. Abraxis BioScience (ABII, Not Rated)
3. Cellceutix (CTIX.OB, Not Rated)
4. Roche (RO.SW, Not Rated)
5. Given Imaging (GIVN, Not Rated)
6. Imaging Diagnostic Systems (IMDS.OB, Not Rated)
7. BioDelivery Sciences International (BDSI, Not Rated)
8. PharmaGap (PHY.BE, Not Rated)
9. Sanofi-Aventis (SNY, Not Rated)
10. Bionovo (BNVI, Not Rated)
11. Febit holding gmbh (Privately Owned)

And many more...

REVIEW IN STEM CELLS

During the week of July 12, 2009 several significant news items pertaining to the stem cell sector were reported including:

1. Pluristem Therapeutics (PSTI, Not Rated) enrolled the first patient in a Phase 1 clinical trial evaluating the safety and efficacy of stem cells derived from human placental tissue for the treatment of critical limb ischemia; and

2. Scientists at the Bedford Stem Cell Research Foundation discovered cell characteristics that may explain important differences between embryonic stem cells and adult stem cells.

ONCOLOGY AND STEM CELL JOURNAL REVIEW

We have searched recent journal articles and highlighted those that warrant investor attention.

ONCOLOGY AND STEM CELL CONFERENCES

We have compiled a conference calendar from July 2009 to December 2009. Data presented at these conferences may affect select companies within the biotech/pharma sector.

WEEK IN REVIEW - ONCOLOGY

1. Oncolytics Biotech Presents Glioma Reovirus Research at ASV Annual Meeting

On July 15, 2009, Oncolytics Biotech (ONCY, Market Outperform) presented a poster entitled “Anti-Tumor Activity of Reovirus is Synergistically Enhanced in Combination with Temozolomide and Low Dose Irradiation in Primary Patient Glioma Xenografts Resistant to Treatment with Either Therapy Alone” at the 28th American Society for Virology (ASV) Annual Meeting. The meeting was held in Vancouver, British Columbia, from July 11-15, 2009.

The poster covers preclinical work using reovirus in combination with low dose irradiation (IR) and temozolomide (TMZ). A panel of glioma cell lines and primary patient glioma explant cells were tested for susceptibility to reovirus, IR, TMZ, or a combination of these therapies in vitro. The combination index analyses revealed moderate to strong synergy in primary patient glioma cells treated with reovirus plus IR and TMZ in vitro. This effect was also seen in vivo in flank tumor xenografts from primary patient tumors resistant to reovirus therapy alone. The investigators concluded that the data supports the use of reovirus in combination with standard radiation and chemotherapies for the treatment of malignant glioma in humans.

2. Oncolytics Biotech Reports Publication of Research on Synergistic Activity of Reovirus and Chemotherapy in NSCLC

On July 16, 2009, Oncolytics Biotech (ONCY, Market Outperform) reported the publication of a study examining reovirus and chemotherapy for the treatment of human non-small cell lung cancer (NSCLC). The paper, entitled “Synergistic Antitumor Activity of Oncolytic Reovirus and Chemotherapeutic Agents in Non-Small Cell Lung Cancer Cells” appears online in *Molecular Cancer*.

The investigators examined in vitro combination effects of reovirus alone and in combination with cisplatin, gemcitabine or vinblastine. When reovirus was used alone, it demonstrated significant cytolytic activity in 7 of 9 NSCLC cell lines examined. The combinations demonstrated strong synergistic effects on cell killing. The combination of reovirus and paclitaxel was invariably synergistic in all cell lines tested, regardless of their level of sensitivity to either agent. The investigators concluded that the oncolytic activity of reovirus can be potentiated by taxanes and other chemotherapeutic agents, and that the reovirus-taxane combination most effectively achieves synergy through accelerated apoptosis triggered by prolonged mitotic arrest.

3. Genta Reports Publication which Confirms Critical Link of LDH Biomarker in Genasense Trials to Overall Survival in Advanced Melanoma

On July 14, 2009, Genta (GETA.OB, Market Perform) reported the publication of a paper that independently confirms the link of a key biomarker to overall survival in patients with advanced melanoma. The biomarker, a tumor-derived enzyme known as lactate dehydrogenase (LDH), is measured by a widely available blood test. Genta’s recently completed Phase 3 trial of Genasense in advanced melanoma, known as AGENDA, specified low-normal LDH as an enrollment criterion. Results for progression-free survival (PFS), a co-primary endpoint of AGENDA, are anticipated in the Fourth Quarter of this year. If positive, the AGENDA results are expected to support global regulatory applications for Genasense in this indication.

A previous randomized Phase 3 trial compared outcomes of patients with advanced melanoma who were treated using standard chemotherapy (dacarbazine) with and without Genasense (oblimersen sodium) Injection, the company’s lead anticancer compound. This trial showed that overall survival (OS) was strongly associated with baseline levels of LDH. While high levels of LDH have long been associated with poor outcome in melanoma and other cancers, the Genasense study showed a significant interaction with treatment outcome in a prospectively stratified randomized trial.

The new publication describes the statistical model that first elucidated an association between increasingly elevated levels of LDH and inferior OS in the previous trial, along with the prospective application of that model to data derived from a recent trial conducted by the European Organization for Research and Treatment of Cancer (EORTC), the largest European oncology cooperative group. The EORTC randomized patients with advanced melanoma to drug combinations that did not employ Genasense and showed no between-treatment differences. The investigators then aggregated data from their study to test the hypothesis generated in the Genasense trial: namely, that OS in advanced melanoma is inversely associated with increasingly elevated levels of LDH.

The new analysis from the EORTC trial confirmed that association and showed results that were highly congruent with observations from the Genasense study. Moreover, the EORTC analysis also confirmed that this association extended into the normal range: specifically, patients with low-normal LDH had extended survival, an observation first established in the Genasense study. Thus, the EORTC analysis provides strong and independent confirmation of the association between survival and LDH levels.

4. CytRx Reports INNO-206 Demonstrates Statistically Significant Tumor Shrinkage in Animal Trial for Breast Cancer

On July 13, 2009, CytRx's (CYTR, Market Outperform) investigational cancer drug INNO-206 caused a dramatic destruction of implanted tumors in an experimental animal model of breast cancer, performing considerably better than the broadly used and generally effective chemotherapeutic drug doxorubicin. In addition to improved efficacy in this animal trial, INNO-206 was comparable in toxicity with doxorubicin based on animal body-weight loss. CytRx has exclusive worldwide rights to INNO-206, a proprietary derivative of doxorubicin. The pro-drug INNO-206 is designed to allow controlled release of doxorubicin and to specifically target the delivery of drug to tumors throughout the body, which could prove more effective and less toxic in cancer patients than doxorubicin. INNO-206 has previously demonstrated safety and tolerability, and optimal dosing has been evaluated, in a prior clinical trial.

In the animal trial – conducted under the direction of INNO-206 inventor Felix Kratz, Ph.D., Department of Medical Oncology, Clinical Research, at the Tumor Biology Center in Freiburg, Germany – human breast tumor cells were implanted in mice with compromised immune systems to avoid tumor rejection. Seven to eight animals were randomly assigned into each of three experimental groups receiving intravenous injections with either a maximum tolerated dose of INNO-206, a maximum tolerated dose of doxorubicin, or a control solution lacking either compound. At the end of the experiment 43 days after implantation, tumors had increased in volume by an average of approximately 2.7-fold in the control group while tumor growth was marginally inhibited in the doxorubicin group; increasing in volume by approximately 1.9-fold in a result that did not reach statistical significance. By contrast, tumors in the group treated with INNO-206 shrank to approximately one-half their initial volume. The decrease in final tumor volume in INNO-206-treated animals was statistically significant ($p < 0.05$) compared to that of either the control or doxorubicin-treated groups.

5. Array BioPharma Initiates Phase 1 Clinical Trial In Cancer with Lead MEK Inhibitor

On July 15, 2009, Array BioPharma (ARRY, Market Outperform) filed an investigational new drug (IND) application with the FDA to initiate a Phase 1 clinical trial in cancer patients with the company's most advanced wholly owned MEK inhibitor, ARRY-162. Recent research confirms that the MEK pathway acts as a central axis in the proliferation of different tumors including melanoma, non-small cell lung, head/neck and pancreatic cancers. Array plans to simultaneously develop ARRY-162 for the treatment of both cancer and inflammatory disease. Array is currently completing a worldwide Phase 2, double-blinded clinical trial with ARRY-162 in 200 patients with active rheumatoid arthritis.

Array believes ARRY-162 is particularly well-suited for use in cancer treatment and has advantages over other MEK inhibitors currently in development, including greater potency, and improved safety and pharmacokinetics. ARRY-162 has been administered to more than 200 patients/volunteers in clinical trials for either safety assessment or the treatment of inflammatory disease. The drug has been well-tolerated and demonstrated significant pharmacodynamic responses in the completed trials. In addition, the company has completed long-term preclinical regulated safety studies and has identified a commercially viable synthetic process and oral formulation for ARRY-162.

The Phase 1 cancer trial will be an open-label, multiple dose study that is designed to determine the maximum tolerated dose and evaluate safety, pharmacokinetics and pharmacodynamics of ARRY-162 following daily oral administration to advanced cancer patients with solid tumors. The trial is expected to commence in the third quarter of this year.

6. OSI Pharmaceuticals Reports New Data from Phase 3 SATURN Study which Shows Tarceva Improves Overall Survival When Used Immediately After Initial Chemotherapy in Patients with Advanced Non-Small Cell Lung Cancer

On July 13, 2009, OSI Pharmaceuticals' (OSIP, Not Rated) and Genentech / Roche's (RO.SW, Not Rated) pivotal Phase 3 study of Tarceva (erlotinib) met a key secondary endpoint of extending overall survival in patients with advanced non-small cell lung cancer (NSCLC) who received Tarceva immediately after initial chemotherapy. A statistically significant improvement in overall survival was seen in this pre-planned final analysis of the total patient population. The new data will be presented during the 13th World Conference on Lung Cancer to be held July 31 to August 4, 2009 in San Francisco.

Treating patients immediately following first-line chemotherapy versus waiting for the cancer to grow or spread before giving additional treatment represents a new approach in advanced NSCLC. The overall survival data will be submitted to the FDA to support the supplemental New Drug Application (sNDA) for use of Tarceva as a first-line maintenance treatment for patients with advanced NSCLC that was submitted on March 17, 2009. The FDA Prescription Drug User Fee Act (PDUFA) review date will be on or about January 18, 2010.

Additionally, Roche (RO.SW, Not Rated), OSI's international collaborator for Tarceva, will submit the overall survival data to the European Medicines Agency (EMA) to support the application for use of Tarceva as a first-line maintenance treatment submitted in March 2009. The U.S. and EU submissions were based on positive data from SATURN that were presented at the 45th Annual Meeting of the American Society of Clinical Oncology (ASCO) on May 31, 2009 in Orlando, Fla. SATURN met the primary endpoint and showed patients with advanced NSCLC who received Tarceva as a first-line maintenance treatment had a 41% improvement in the time they lived without the disease advancing (progression-free survival or PFS) compared to placebo (hazard ratio=0.71; 29% reduction in the risk of cancer progression or death). The safety results were consistent with what has been seen previously and there were no new or unexpected safety signals in the study. The most commonly reported adverse events in patients who received Tarceva were rash (49%, 213/438) and diarrhea (20%, 88/438).

7. Health Discovery Reports Publication of Peer Reviewed Scientific Paper Demonstrating the Clinical Validity of the Four-Gene Prostate Cancer Test Licensed to Abbott, Quest Diagnostics and Clariant

On July 14, 2009, Health Discovery's (HDVY.OB, Not Rated) scientific paper entitled "A Four-Gene Expression Signature for Prostate Cancer Cells Consisting of UAP1, PDLIM5, IMPDH2, and HSPD1" has successfully completed the peer review process and has been approved for publication in the August issue of UroToday International Journal. This publication demonstrates the clinical validity of the four-gene prostate cancer test for identifying clinically significant prostate cancer licensed for commercialization by HDC to Abbott (ABT, Not Rated), Quest Diagnostics (DGX, Not Rated), and Clariant (CLRT, Not Rated).

8. Watson Pharmaceuticals Receives a Complete Response Letter for 24-Week Formulation of TRELSTAR (Triptorelin Pamoate) NDA from FDA

On July 14, 2009, Watson Pharmaceuticals (WPI, Not Rated) received a Complete Response Letter from the FDA for the company's New Drug Application for TRELSTAR 22.5 mg (triptorelin pamoate for injectable suspension), a 24-week formulation of TRELSTAR for the palliative treatment of advanced prostate cancer. The TRELSTAR NDA was prepared in cooperation with Debiopharm Group (Privately Owned).

According to the letter, the FDA has requested clarifications related to the clinical testing of the product, additional information regarding the chemistry, manufacturing and controls (CMC) of the product and other components, and information related to third party manufacturing. Watson is working to ensure the requested information is provided to the FDA expeditiously. TRELSTAR 22.5 mg builds on Watson's long-standing track record in prostate cancer and expanding urology franchise. The new, longer-acting formulation of TRELSTAR is designed to be conveniently administered and to continuously suppress the production of testosterone in men with advanced prostate cancer for 24 weeks. TRELSTAR is an already proven therapy with established efficacy and safety in two formulations - a four-week formulation (TRELSTAR DEPOT) and a 12-week formulation (TRELSTAR LA).

9. Abraxis Bioscience Completes Enrollment of Pivotal Phase 3 Advanced Lung Cancer Study Evaluating Abraxane vs. Taxol

On July 13, 2009, Abraxis BioScience (ABII, Not Rated) completed patient enrollment in a pivotal, Phase 3 clinical study comparing the company's chemotherapy agent ABRAXANE for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin bound) with Taxol (paclitaxel) injection, both in combination with carboplatin, in the first-line treatment of patients with advanced non-small cell lung cancer (NSCLC). The study, which is being conducted at 111 sites globally, includes 1,050 patients and is being led by principal investigator Dr. Mark Socinski at the University of North Carolina Lineberger Comprehensive Cancer Center. It is one of the largest NSCLC clinical studies to complete enrollment.

ABRAXANE is currently approved for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within six months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated. NSCLC comprises 85% to 90% of lung cancers.

The Phase 3 study involves 1,050 patients randomized in a one-to-one ratio to two treatment arms: patients in Arm A (n=525) receive ABRAXANE 100 mg/m² weekly plus carboplatin AUC 6 on Day 1 of a three-week treatment cycle; and patients in Arm B (n=525) receive Taxol 200 mg/m² plus carboplatin AUC 6 on Day 1 of a three-week treatment cycle. The primary study endpoint is disease response, measured as complete and partial responses as defined by RECIST (Response Evaluation Criteria in Solid Tumors). Secondary study endpoints include: safety and tolerability; disease control rate and duration of response; progression-free survival (PFS); patient survival; and assessments of ABRAXANE efficacy correlated with specific tumor biomarkers, including secreted protein acidic and rich in cysteine (SPARC).

10. Cellceutix Reports Kevetrin Animal Model Testing Success against Multi-Drug Resistant Lung Cancer Cell Lines

On July 13, 2009, Cellceutix (CTIX.OB, Not Rated) completed a series of animal model tests on two multi-drug resistant non-small-cell lung carcinoma human cell lines, A549 and NCI-H1975, using the proprietary pharmaceutical compound Kevetrin. In each cell line, tumor volume was reduced by more than 90% and tumor growth was delayed by more than 100%. In addition, both the tumor volume reduction and the tumor growth delay were greater in each cell line with Kevetrin than with paclitaxel (brand name Taxol(r)) (p<0.01). Each experiment was repeated in order to increase the level of confidence in the results. The results in the repeated experiments were similar to those in the initial tests.

In the experiments, nude mice were implanted subcutaneously with human tumor cells. Groups of 10 mice bearing A549 or NCI-H1975 tumors were treated with Kevetrin or paclitaxel alone or acted as controls. Neither Kevetrin nor paclitaxel produced significant weight loss in the study subjects. In the NCI-H1975 tumors, Kevetrin significantly delayed tumor growth an average of 28 days (156%) compared to controls and 14 additional days when compared to the paclitaxel treated mice. When measured at day 25, Kevetrin significantly reduced tumor volumes an average of 2,827 mm (91%) compared to controls, while paclitaxel reduced tumor volumes an average of 2,535 mm (82%) compared to controls. In the A549 tumors, Kevetrin significantly delayed tumor growth an average of 30 days (111%) compared to controls and 27 additional days when compared to the paclitaxel treated mice. When measured at day 33, Kevetrin significantly reduced tumor volumes an average of 2,138 mm (97%) compared to controls, while paclitaxel reduced tumor volumes an average of 53 mm (2%).

11. Abbott and GSK to Collaborate on Molecular Diagnostic Test to Select Candidate Patients for Future Cancer Immunotherapy

On July 13, 2009, Abbott (ABT, Not Rated) entered into an agreement with GlaxoSmithKline (GSK, Not Rated) to develop an automated molecular diagnostic test, based on polymerase chain reaction (PCR) technology, intended to screen non-small cell lung cancer (NSCLC) tumors for expression of the MAGE-A3 antigen. GSK's MAGE-A3 ASCI (Antigen Specific Cancer Immunotherapy) candidate is currently being evaluated as an adjuvant treatment in resected NSCLC in the Phase 3 clinical study MAGRIT, the largest lung cancer treatment study ever conducted. To be eligible to receive GSK's MAGE A3 ASCI, patients must have MAGE-A3 expressing NSCLC tumors. MAGE-A3 is a tumor-specific antigen that is expressed in non-small cell lung cancer and a wide variety of other cancers, but not in normal cells. Of note, GSK's MAGE-A3 ASCI vaccine contains Antigenics' (AGEN, Market Perform) QS-21 adjuvant.

Under terms of the agreement, Abbott, in conjunction with GSK, will develop and commercialize a PCR test designed to detect MAGE A3 for use on the Abbott m2000 automated instrument system. Currently, there are no nucleic acid based tests approved by the FDA for use in identifying patients who may derive treatment benefits from targeted non-small cell lung cancer therapies.

GSK's ASCIs represent a novel class of medicines designed to train the immune system to recognize and eliminate cancer cells in a highly specific manner. These novel cancer immunotherapeutics combine tumor antigens, delivered as purified recombinant proteins, and GSK's proprietary Adjuvant Systems, which are specific combinations of immunostimulating compounds selected to increase the anti-tumor immune response. ASCIs are being investigated in the clinic to support their use to reduce the risk of tumor recurrence following surgery, or to impact tumor growth in an early metastatic setting. The highly specific mode of action of GSK's ASCIs allows development of diagnostic tools to aid in selecting patients eligible for the treatment, depending on the expression of the tumor antigens.

MAGE-A3 is a tumor-specific antigen that is expressed in a large variety of cancers, including melanoma, non-small cell lung cancer, head and neck cancer, bladder cancer, with no expression in normal cells. Expression of the MAGE-A3 gene has been observed in testicular cells but without antigen presentation capabilities. MAGE-A3 protein has been in-licensed by GSK from the Ludwig Institute for Cancer Research, the largest international academic institute dedicated to understanding and controlling cancer. MAGE-A3 ASCI is an investigational compound and it is not approved for use in any indication in any country at this time.

12. Roche Drug Blocks Common Cancer Pathway in Mice

On July 14, 2009, scientists discovered that an experimental drug being developed by Roche (RO.SW, Not Rated) blocks a common biological pathway linked to the spread of many cancers and has proved highly effective in mice. The compound GDC-0941 was originally pioneered by British biotechnology company Piramed (Privately Owned). It is currently in Phase 1 clinical studies, the first round of testing in humans, in Britain and the US. A study published in the journal *Molecular Cancer Therapeutics* showed the drug reduced the growth of glioblastoma -- the most common form of brain tumor - in mice by up to 98% and decreased the growth of ovarian tumors 80%. Scientists also found the drug worked against a number of cell lines derived from other human cancers. GDC-0941 works by blocking the phosphatidylinositide 3-kinase (PI3) pathway which is often hijacked by cancer cells, enabling them to grow and spread.

13. Veridex Reports Monitoring Circulating Tumor Cells with the CellSearch System which Can Predict Prognosis in Metastatic Breast Cancer

On July 13, 2009, Veridex, subsidiary of Johnson & Johnson (JNJ, Not Rated) published a study in the *Journal of Clinical Oncology* demonstrating that measuring the change in circulating tumor cell (CTC) count can accurately predict the prognosis and survival in patients with metastatic breast cancer (MBC). The retrospective study compared how well CTCs and a more sensitive than conventional modality, fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT), predicted survival in MBC patients on standard therapies. The comparison showed that both technologies significantly correlate to overall MBC patient survival ($p < 0.001$ for CTCs and $p = 0.001$ for FDG-PET/CT). However, a CTC count of five or more could better predict the prognosis and survival in MBC patients.

The CellSearch System is the first 510(k) diagnostic test used to automate the capture and detection of CTCs, tumor cells that have detached from solid tumors and entered the patient's blood. CTCs and FDG-PET/CT are two of the most promising new tools for therapeutic monitoring in patients with MBC. The number of CTCs identified in patients with MBC is related to patient prognosis; a high number of CTCs at any time during treatment is associated with poor prognosis.

14. Quantum Immunologics Reports the Completion of First Clinical Treatment Injection

On July 14, 2009, Quantum Immunologics (Privately Owned) treated the first patient in the company's breast cancer trial evaluating a dendritic cell therapy. QI is currently sponsoring and conducting an FDA-authorized Phase 1/2 clinical trial testing the safety and efficacy of the company's immunotherapy on 27 Stage 4 breast cancer patients who have failed conventional therapy. According to Mr. Chuck Broes, CEO of QI, This achievement marks a significant milestone in the life cycle of our company, and we look forward with great hope and commitment as we aspire to a successful trial outcome.

The clinical trials involve the use of dendritic cell therapy using the oncofetal antigen (OFA), or iLRP -- immature Laminin Receptor Protein, as a cancer antigen (a protein found on cancer cells that can be targeted by the body's own immune system) found in many tumor cell lines or fetal tissue, but absent on normal, healthy tissue. QI believes that the OFA antigen can serve as a unique, valuable and promising antigen for individualized breast cancer immunotherapy.

Centered in Mobile, AL, QI's clinical trial is designed around the use of QI's proprietary dendritic cell therapy, which employs OFA to recruit the patient's own immune system to target and attack the cancer cells with the intent to improve patient survivability and quality of life. Each patient will receive three monthly injections of the patient's own dendritic cells that have been sensitized to OFA. It is anticipated that once the sensitized cells are injected back into the patient, the patient's T-cells will locate the OFA found on the patient's cancer cells, thereby generating an immune response with the goal of killing the cancer cells and preventing further spread of the disease.

15. Centocor Ortho Biotech Reports FDA ODAC Opinion Regarding Trabectedin for Relapsed Ovarian Cancer

On July 15, 2009, Centocor Ortho Biotech, a subsidiary of Johnson & Johnson (JNJ, Not Rated), reported that the FDA Oncologic Drugs Advisory Committee determined that the combination of trabectedin when administered with DOXIL (doxorubicin HCl liposome injection) did not provide a sufficient benefit-risk profile for the treatment of relapsed ovarian cancer. Ovarian cancer is difficult to treat and the disease often recurs in patients who previously have been treated with platinum-based therapy, underscoring the need for non-platinum treatment options. Centocor Ortho Biotech Products continues to believe trabectedin has an important role in the treatment of recurrent ovarian cancer. The company remains committed to working with the FDA to address the committee's concerns. The committee provides non-binding recommendations based on its evaluation. The final decision regarding approval of the drug will be made by the FDA.

16. Panel Votes to Reject J&J, Zeltia Cancer Drug

On July 15, 2009, Johnson & Johnson (JNJ, Not Rated) and Zeltia SA's (ZEL.MC, Not Rated) drug Yondelis for the treatment of ovarian cancer was recommended against approval, as risks of heart and liver toxicity outweigh the drug's limited ability to keep the disease in check, an advisory panel to the FDA concluded by a vote of 14-1. The FDA typically, but not always, abides by recommendations of advisory panels. While the rejection is relatively inconsequential for giant healthcare conglomerate J&J, it represents a major setback for Spanish biotechnology company Zeltia.

Yondelis, known chemically as trabectedin, was approved for the treatment of recurrent ovarian cancer in the Philippines last week, raising hopes it would get a favorable reception from the U.S. advisory committee. But panelists overwhelmingly felt that the modest 6-week benefit in progression-free survival (PFS) shown in a pivotal late-stage clinical trial did not justify approval. The trial tested Yondelis plus Doxil chemotherapy versus Doxil alone. J&J's Centocor Ortho Biotech unit and Zeltia stated they were committed to working with the FDA to address the committee's concerns.

The panel was asked to vote on whether adding trabectedin to Doxil represented a favorable benefit/risk ratio in this patient population. While the risk bar is considerably higher for drugs for extremely sick advanced cancer patients than those for chronic diseases, a notable increase in severe and life-threatening adverse events in the Yondelis treatment group swung the vote against the drug. Those included more than twice as many incidents of pulmonary embolism - a potentially lethal blockage of the arteries to the lungs. Cardiac adverse events were three times higher in the Yondelis treatment group, and liver enzyme increases that can lead to liver damage were much higher. Panelists also expressed skepticism about the value of progression-free survival - the time it takes for the cancer to worsen - as a primary goal for a type of cancer for which other approved therapies exist. Many seemed willing to revisit the viability of the drug once overall survival data is known. Final overall survival data will not be available for about 18 to 24 months, the companies stated. Interim analysis, which may not be indicative of final data, has so far found no significant difference in overall survival.

Company representatives argued that PFS was a clinically meaningful goal and stated the drug could be an important treatment option for relapsed ovarian cancer patients who cannot tolerate platinum-based chemotherapies. The hearing included emotional testimony from a cancer survivor, who credited Yondelis for her recovery. She had suffered from a different type of advanced cancer that failed to respond to treatment with Eli Lilly and (LLY, Not Rated) Gemzar or Pfizer (PFE, Not Rated) Sutent, but stated her tumors disappeared after being treated with Yondelis in a clinical trial. The committee also voted 13-0 to reject a separate application from J&J for wider use of Doxil. Panel members stated benefits did not outweigh risks when a combination of Doxil and an older drug, docetaxel, was used as a first-choice treatment for advanced breast cancer patients.

17. Given Imaging Reports Results of Multi-Center Study on First-Generation PillCam COLON Published in New England Journal of Medicine

On July 15, 2009, Given Imaging's (GIVN, Not Rated) results from a prospective multi-center study comparing the performance of PillCam COLON capsule endoscopy with optical colonoscopy for the detection of colorectal polyps and cancer were published in issue of the New England Journal of Medicine. The study's authors concluded that while this first-generation PillCam COLON's sensitivity for detecting colonic lesions was lower than colonoscopy, colon capsule endoscopy is a safe way to visualize the colon that obviates the need for sedation, intubation or air insufflation. The multi-center study enrolled 328 patients between January 2006 and July 2007 with known or suspected colonic disease. The primary endpoint of the study was detection of colorectal polyps and cancer. As with colonoscopy, PillCam COLON's sensitivity was significantly higher in patients with adequate cleansing levels. Researchers computed sensitivity and specificity of capsule endoscopy for polyps, advanced adenoma and cancer. The sensitivity for detecting large polyps (greater than or equal to 6 mm) and advanced adenomas was 64% and 73%, respectively and specificity was 84% and 79%, respectively.

Given Imaging received the CE Mark for the first-generation version of PillCam COLON in 2006, which is now available commercially in Europe, Asia, Latin America, Canada and Australia. PillCam COLON is not approved for use in the U.S. Given Imaging is currently advancing a second-generation PillCam COLON capsule through clinical trials in Israel.

18. Imaging Diagnostic Systems Reports German University Study Aimed at Expanding the Clinical Utility of CTLM Laser Breast Imaging System

On July 15, 2009, Imaging Diagnostic Systems (IMDS.OB, Not Rated) initiated a breast cancer imaging study at the Charité, Medical University in Berlin, Germany. The study will examine the potential role of the model 1020 CTLM laser breast imaging system as an enhanced breast cancer screening tool when used in combination with the fluorescent dye, Indocyanine Green (ICG). The study will be conducted at the Campus Virchow-Klinikum and the principal investigator is radiologist Dr. Alexander Poellinger.

The ICG fluorescent dye has a distribution pattern in the human body similar to that of extracellular MRI and CT contrast agents and it is already approved for other medical applications. The IDSI model 1020 CTLM scanner that will be used in the study has been specially modified to be able to both excite the ICG dye and image its fluorescence in breast tissue. Following injection of ICG into the patient, the scanner produces 3D images of the localized concentration of the dye. These images are expected to show increased extravasation and accumulation of the dye in malignant tissue. IDSI users have performed over 15,000 CT Laser Mammography (CTLM) clinical cases worldwide

19. BioDelivery Sciences and Meda Announce FDA Approval of ONSOLIS

On July 16, 2009, BioDelivery Sciences International (BDSI, Not Rated) and Meda AB (MDABF.PK, Not Rated) received approval from the FDA to market ONSOLIS (fentanyl buccal soluble film), formerly referred to as BEMA Fentanyl, for the management of breakthrough pain (BTP) in patients with cancer, 18 years of age and older, who are already receiving and who are tolerant to opioid therapy for their underlying persistent cancer pain. ONSOLIS is the first product to utilize the company's proprietary BioErodible MucoAdhesive (BEMA) drug delivery technology, which consists of a small, dissolvable, polymer film for application to the buccal mucosa (inner lining of the cheek).

ONSOLIS is anticipated to be available in the fourth quarter of 2009 and will be commercialized in the U.S. by Meda Pharmaceuticals; the U.S. subsidiary of Meda AB. Meda is the company's commercialization partner for the product worldwide, with the exception of Taiwan and South Korea, the rights to which remain with BDSI.

20. PharmaGap Provides Guidance on Cancer Drug Testing Timetable

On July 17, 2009, PharmaGap (PHY.BE, Not Rated) updated guidance on the anticipated timing of the receipt of test results from the previously reported testing initiatives for the company's lead cancer drug, PhG-alpha-1, at the U.S. National Cancer Institute (NCI) and the Ottawa Hospital Research Institute (OHRI). Results from these tests will be reported once received and have been fully analyzed and understood by the company's scientific team and outside drug development advisors. The company has been informed by the OHRI that initial results from the OHRI test program, being the effect of PhG-alpha-1 at a range of dose concentrations on the growth rate of ovarian cancer cells are expected in or prior to the first week of August 2009, following completion of a full statistical analysis.

Additionally, the company is advised by the OHRI that the second phase of the test program, which will evaluate the effect of PhG-alpha-1 on cell invasion and mobility in a number of different ovarian cancer cell lines, is about to commence. The company now anticipates that results of testing of PhG-alpha-1 at the NCI using the NCI-60 cancer cell line panel will be received by the company in mid August 2009. The NCI-60 protocol tests a wide variety of cancers, including breast, colorectal, lung, ovarian, kidney, prostate, leukemia and various central nervous system cancers and melanomas. The company will provide updates and release of all material test results as soon as possible following receipt of test data and analysis by company scientists and the Clinical Advisory Group.

21. Sanofi-Aventis Reports BSI-201 Enters Phase 3 in Metastatic Triple Negative Breast Cancer

On July 17, 2009, Sanofi-Aventis (SNY, Not Rated) and a wholly owned subsidiary, BiPar Sciences, initiated a pivotal Phase 3 trial evaluating BSI-201 in combination with chemotherapy for the treatment of metastatic triple-negative breast cancer (mTNBC), defined by tumors lacking expression of estrogen, progesterone receptors and without over-expression of HER2. BSI-201 is a novel, investigational, targeted therapy which inhibits poly (ADP-ribose) polymerase (PARP1), an enzyme involved in DNA damage repair.

The Phase 3 trial is a multi-center, randomized trial designed to evaluate the safety and efficacy of BSI-201 when combined with gemcitabine and carboplatin (GC) in women with mTNBC. A total of 420 mTNBC patients, who have received 0-2 prior therapies in the metastatic setting, will be randomized to receive GC with or without BSI-201. The co-primary objectives of this study are to assess improvement in progression-free survival and overall survival. The secondary objectives are to assess objective response rate and safety. An estimated 60-75 sites will be distributed throughout the United States. Importantly, this trial will have a crossover provision that will ensure that all patients enrolled in the BSI-201 Phase 3 clinical trial have the potential opportunity to receive BSI-201 (patients randomly assigned to the control arm may receive BSI-201 upon disease progression).

The decision to commence with the Phase 3 study was made based on Phase 2 study results presented during the Plenary Session of the American Society of Clinical Oncology (ASCO) annual conference on May 31, 2009. The Phase 2 clinical trial involved 116 women with metastatic TNBC who were randomly assigned to receive GC in combination with the investigational agent BSI-201 or GC alone. Approximately 62% of patients receiving BSI-201 in combination with GC showed clinical benefit, compared with 21% in the group receiving chemotherapy alone ($p=0.0002$). Tumor response (complete or partial response) was observed in 48% of patients who received BSI-201 combined with chemotherapy, whereas patients receiving chemotherapy alone showed a response rate of 16%. Women who received BSI-201 had a median progression-free survival of 6.9 months and overall survival of 9.2 months compared with 3.3 and 5.7 months, respectively, for women who received chemotherapy alone. The hazard ratios for progression-free survival and overall survival were 0.342 ($p<0.0001$) and 0.348 ($p=0.0005$), respectively.

The most common severe (grades 3 and 4) side effects included neutropenia [25/57 in patients treated with GC and BSI-201; 31/59 patients treated with GC alone], thrombocytopenia and anemia. No febrile neutropenia was observed in patients receiving BSI-201 combined with chemotherapy. BSI-201 did not add to the frequency or severity of adverse events associated with chemotherapy.

Among other investigational PARP inhibitors in the industry, BSI-201 is the furthest along in clinical development in metastatic TNBC. BSI-201 is currently being evaluated for the potential to enhance the effect of chemotherapy-induced DNA damage.

22. Bionovo Reports Estrogen Receptor Beta Selective Drugs Have Unique Gene Expression and Cell Type Specificity

On July 17, 2009, Bionovo's (BNVI, Not Rated) study of gene regulation in multiple cell lines by several of the company's estrogen receptor beta (ERb) candidates was slated for publication in Public Library of Science One. The publication describes the analyses of three distinct classes of ERb selective drugs. The study determines the relative ER selectivity and pattern of gene expression of the three classes of ERb selective compounds compared to the natural hormone estradiol, which non-selectively regulates both ERa and ERb. The most significant finding in the study was that the ERb-selective compounds regulate a number of genes differently than estradiol, a hormone therapy commonly used to treat women's health issues. This discovery indicates that ERb agonists might be safer than current estrogens used in hormone therapy. The study also demonstrates the cell type selectivity of Menerba (MF101) and Liquiritigenin, two of Bionovo's ERb selective drugs, in different cell types. These compelling findings will serve as a strong impetus for Bionovo to continue investigating the unique abilities of their drugs to safely and effectively treat menopausal symptoms.

23. Febit Reports First Catalog Cancer Biochip for Sequence Capture

On July 13, 2009, Febit holding gmbh (Privately Owned) launched the first human cancer biochip for HybSelect, febit's highly automated technology for sequence capture, enabling targeted Next-Generation Sequencing (NGS). The new catalog cancer biochip features 115 important genes which are reported to be associated with common types of cancer by the Wellcome Trust Sanger Institute.

The availability of the automated HybSelect technology supports large-cohort NGS studies that aim to elucidate complex diseases such as cancer, drive forward personalized medicine and investigate new therapeutic delivery models. Febit will stay at the forefront of cancer research and include key research study results in the next generation of febit's cancer biochips. A 2Mb exon cancer biochip will be available this summer and a 30Mb biochip is planned already for release in 2010.

Multiplex NGS studies based on sequence capture can be supplemented by other molecular fingerprinting methods, including mRNA and miRNA expression profiling. Both of these high-throughput methods can be performed using the same febit technology as HybSelect, thereby reducing capital expenditures and minimizing experimental variation. HybSelect and expression profiling can easily be performed in the researcher's lab operating a Geniom RT Analyzer from febit or by using febit's convenient Analytical Services.

WEEK IN REVIEW - STEM CELL TECHNOLOGY

1. Pluristem Therapeutics Reports Placenta-Derived Stem Cells Tested in Phase 1 Trial for PAD Treatment

On July 18, 2009, Pluristem Therapeutics (PSTI, Not Rated) enrolled the first patient in a Phase 1 clinical trial evaluating the safety and efficacy of stem cells derived from human placental tissue for the treatment of critical limb ischemia (CLI), the end-stage of peripheral artery disease (PAD). The therapy is being evaluated in a clinical trial at 2 German medical facilities. The first patient was enrolled at the Franziskus-Krankenhaus Hospital, Berlin. The initiation of this study follows the recent approval of the company's Clinical Trial Application (CTA) to begin clinical trials with allogeneic placental-derived adherent stromal cell product, termed PLX-PAD. PLX-PAD by the Paul Ehrlich Institute (PEI), the German competent authority in the European Union. The Phase 1 study is designed to evaluate the safety of PLX-PAD in patients with CLI. A total of up to 15 adults with the disease will be included in the trial which is being conducted at the Franziskus-Krankenhaus Hospital and Charit  - Universit tsmedizin Hospital, Berlin. Pluristem is dedicated to the commercialization of unrelated donor-patient (allogeneic) cell therapy products for the treatment of several severe degenerative, ischemic and autoimmune disorders. PLX-PAD is a first-in-human placental-derived mesenchymal-like stromal cell product that has received FDA and IMPD clearance and is being investigated in a Phase 1 clinical trial.

2. New Finding May Provide Key to Multiplication of Autologous Stem Cells

On July 17, 2009, stem cell researchers discovered cell characteristics that may explain important differences between embryonic stem cells and adult stem cells. Dr. Ann Kiessling of the Bedford Stem Cell Research Foundation discovered that early human embryo cells surprisingly express CLOCK and other circadian genes that other human cells growing in laboratories did not. Although scientists have recently become aware that human tissues have a circadian oscillator that cycles every 24 hours, in phase with the master circadian pacemaker in the brain that responds to light and dark, early embryos seemed too small to function like a tissue.

Kiessling also discovered that the RB gene, a powerful cell blockade, was turned off in the early embryo cells. This was also a surprise because RB is a well-studied blockade that prevents cells from multiplying unless needed and stimulated by growth factors. The lack of RB in the early embryo cells, in combination with the circadian oscillator, are unique characteristics that together enable independent, continuous cell duplication. Developing conditions to inhibit RB and support the circadian oscillator may be the combination long sought by scientists to multiply adult stem cells to the trillions needed for therapies for diseases such as heart failure, diabetes, Parkinson's disease, spinal cord injury, AIDS and cancer. The work was the result of collaboration between Kiessling's team of scientists at the Mass.-based Bedford Stem Cell Research Foundation with a team of clinician scientists at the University of Athens in Greece. The unique work was made possible, according to Kiessling, through the use of cutting-edge microarray technology. BRF scientists examined expression of 44,000 gene elements in discarded early-stage (8 cells of development) human embryos.

WEEKLY JOURNAL REVIEW IN ONCOLOGY

FROM JULY 15, 2009, VOLUME 69, ISSUE 14 OF *CANCER RESEARCH*

1. Polyps Wrap Mast Cells and Treg within Tumorigenic Tentacles, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5619-5622

ABSTRACT: Gounaris and colleagues describe a previously unrecognized cross-talk between mast cells and Treg in colon adenomatous polyposis (Gounaris et al., *Cancer Res* 2009;69:5490–7). Adoptively transferred Treg suppress the focal mastocytosis that fosters tumor initiation and progression. In contrast, endogenous Treg, which abundantly infiltrate polyps, show proinflammatory activity under unknown microenvironmental cues that promote mast cell differentiation and expansion. Compartmentalized Treg plasticity seems to be a key factor in establishing the optimal milieu for cancer development in the intestines. Treg partnership with mast cells recapitulates the complexity of innate-adaptive networks characterizing gut inflammation and represents a novel target for cancer immunotherapy.

2. Platelets: Guardians of Tumor Vasculature, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5623-5626

ABSTRACT: Solid tumors generate a prothrombotic environment capable of platelet activation. Recent findings indicate that the activated platelets are crucial regulators of tumor vascular homeostasis in that they prevent tumor hemorrhage. Surprisingly, this effect is independent of platelets' capacity to form thrombi and instead relies on the secretion of their granule content. Thus, targeting platelet secretory activities may represent a new approach to specifically destabilize tumor vasculature.

3. The Emerging Role of EpCAM in Cancer and Stem Cell Signaling, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5627-5629

ABSTRACT: Initially discovered as a dominant antigen on colon carcinomas, the epithelial cell adhesion molecule (EpCAM) was considered a mere cell adhesion molecule and reliable surface-binding site for therapeutic antibodies. Recent findings can better explain the relevance of EpCAM's high-level expression on human cancers and cancer propagating cells, and its negative prognostic potential for survival of patients with certain cancers. EpCAM has oncogenic potential and is activated by release of its intracellular domain, which can signal into the cell nucleus by engagement of elements of the wnt pathway.

4. Sample Type Bias in the Analysis of Cancer Genomes, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5630-5633

ABSTRACT: There is widespread agreement that cancer gene discovery requires high-quality tumor samples. However, whether primary tumors or cultured samples are superior for cancer genomics has been a longstanding subject of debate. This debate has recently become more important because federally funded cancer genomics has been centralized under The Cancer Genome Atlas, which has chosen to focus exclusively on primary tumors. Here, we provide a data-driven "perspective" on the effect of sample type selection on cancer genomics research. We show that, in the case of glioblastoma multiforme, primary tumors and xenografts are best for the identification of amplifications, whereas xenografts and cell lines are superior for the identification of homozygous deletions. We also note that many of the most important oncogenes and tumor suppressor genes have been discovered through the use of cell lines and xenografts, and highlight the lack of published evidence supporting the dogma that ex vivo culture generates artifactual genetic lesions. Based on this analysis, we suggest that cancer genomics projects such as The Cancer Genome Atlas should include a variety of sample types such as xenografts and cell lines in their integrated genomic analysis of cancer.

5. Protein Kinase D Regulates Cell Migration by Direct Phosphorylation of the Cofilin Phosphatase Slingshot 1 Like, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5634-5638

ABSTRACT: Protein kinase D (PKD) has been identified as a negative regulator of epithelial cell migration; however, its molecular substrates and downstream signaling pathways that mediate this activity have remained elusive. In this study, we provide evidence that the cofilin phosphatase slingshot 1 like (SSH1L), an important regulator of the complex actin remodeling machinery, is a novel *in vivo* PKD substrate. PKD-mediated phosphorylation of serines 937 and 978 regulates SSH1L subcellular localization by binding of 14-3-3 proteins and thus impacts the control of local cofilin activation and actin remodeling during cell migration. In line with this, we show that the loss of PKD decreases cofilin phosphorylation, induces a more spread cell morphology, and stimulates chemotactic migration of breast cancer cells in an SSH1L-dependent fashion. Our data thus identify PKD as a central regulator of the cofilin signaling network via direct phosphorylation and regulation of SSH1L.

6. MicroRNA-661, a c/EBP α Target, Inhibits Metastatic Tumor Antigen 1 and Regulates Its Functions, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5639-5642

ABSTRACT: MicroRNAs (miR) have been identified as posttranscriptional modifiers of target gene regulation and control the expression of gene products important in cancer progression. Here, we show that miR-661 inhibits the expression of metastatic tumor antigen 1 (MTA1), a widely up-regulated gene product in human cancer, by targeting the 3' untranslated region (UTR) of MTA1 mRNA. We found that endogenous miR-661 expression was positively regulated by the c/EBP α transcription factor, which is down-regulated during cancer progression. c/EBP α directly interacted with the miR-661 chromatin and bound to miR-661 putative promoter that contains a c/EBP α -consensus motif. In addition, we found that the level of MTA1 protein was progressively up-regulated, whereas that of miR-661 and its activator, c/EBP α , were down-regulated in a breast cancer progression model consisting of MCF-10A cell lines whose phenotypes ranged from noninvasive to highly invasive. c/EBP α expression in breast cancer cells resulted in increased miR-661 expression and reduced MTA1 3'UTR-luciferase activity and MTA1 protein level. We also provide evidence that the introduction of miR-661 inhibited the motility, invasiveness, anchorage-independent growth, and tumorigenicity of invasive breast cancer cells. We believe our findings show for the first time that c/EBP α regulates the level of miR-661 and in turn modifies the functions of the miR661-MTA1 pathway in human cancer cells. Based on these findings, we suggest that miR-661 be further investigated for therapeutic use in down-regulating the expression of MTA1 in cancer cells.

7. Tumor Necrosis Factor- α and Interleukin-1 Antagonists Alleviate Inflammatory Skin Changes Associated with Epidermal Growth Factor Receptor Antibody Therapy in Mice, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5643-5647

ABSTRACT: Cancer patients receiving epidermal growth factor receptor (EGFR) antibody therapy often experience an acneiform rash of uncertain etiology in skin regions rich in pilosebaceous units. Currently, this condition is treated symptomatically with very limited, often anecdotal success. Here, we show that a monoclonal antibody targeting murine EGFR, ME1, caused a neutrophil-rich hair follicle inflammation in mice, similar to that reported in patients. This effect was preceded by the appearance of lipid-filled hair follicle distensions adjacent to enlarged sebaceous glands. The cytokine tumor necrosis factor- α (TNF α), localized immunohistochemically to this affected region of the pilosebaceous unit, was specifically up-regulated by ME1 in skin but not in other tissues examined. Moreover, skin inflammation was reduced by cotreatment with the TNF α signaling inhibitor, etanercept, indicating the involvement of TNF α in this inflammatory process. Interleukin-1, a cytokine that frequently acts in concert with TNF α , is also involved in this process given the efficacy of the interleukin-1 antagonist Kineret. Our results provide a mechanistic framework to develop evidence-based trials for EGFR antibody-induced skin rash in patients with cancer.

8. Expression of an Exogenous Human Oct-4 Promoter Identifies Tumor-Initiating Cells in Osteosarcoma, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5648-5655

ABSTRACT: We explored the nature of the tumor-initiating cell in osteosarcoma, a bone malignancy that predominately occurs in children. Previously, we observed expression of Oct-4, an embryonal transcriptional regulator, in osteosarcoma cell cultures and tissues. To examine the relationship between Oct-4 and tumorigenesis, cells from an osteosarcoma biopsy (OS521) were stably transfected with a plasmid containing the human Oct-4 promoter driving a green fluorescent protein (GFP) reporter to generate the transgenic line OS521Oct-4p. In culture, only ~24% of the OS521Oct-4p cells were capable of activating the transgenic Oct-4 promoter; yet, xenograft tumors generated in NOD/SCID mice contained ~67% GFP⁺ cells, which selectively expressed the mesenchymal stem cell-associated surface antigens CD105 and ICAM-1. Comparison of the tumor-forming capacity of GFP-enriched and GFP-depleted cell fractions revealed that the GFP-enriched fractions were at least 100-fold more tumorigenic, capable of forming tumors at doses of <300 cells, and formed metastases in the lung. Clonal populations derived from a single Oct-4/GFP⁺ cell were capable of forming tumors heterogeneous for Oct-4/GFP expression. These data are consistent with the cancer stem cell model of tumorigenesis in osteosarcoma and implicate a functional link between the capacity to activate an exogenous Oct-4 promoter and tumor formation. This osteosarcoma tumor-initiating cell appears highly proliferic and constitutes a majority of the cell population in a primary xenograft tumor, which may provide a biological basis for the particular virulence of this type of cancer.

9. Human Papillomavirus E7 Oncoprotein Overrides the Tumor Suppressor Activity of p21^{Cip1} in Cervical Carcinogenesis, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5656-5663

ABSTRACT: The E7 oncoprotein of the high-risk human papillomaviruses (HPV) is thought to contribute to cervical carcinogenesis at least in part by abrogating cell cycle regulation. E7 can dysregulate the cell cycle through its interaction with several cellular proteins including the retinoblastoma suppressor protein pRb, as well as the cyclin-dependent kinase inhibitor p21^{Cip1}. Inactivation of pRb in cervical epithelia is not sufficient to explain the ability of E7 to cause cervical cancers in transgenic mice. In the current study, we focused on the role of p21^{Cip1} in cervical cancer. Cervical disease was significantly increased in p21^{-/-} mice compared with p21^{+/+} mice, showing that p21^{Cip1} can function as a tumor suppressor in this tissue. Importantly, the ability of E7 to induce cervical cancers was not significantly enhanced on the p21-null background, consistent with the hypothesis that the ability of E7 to inhibit p21^{Cip1} contributes to its carcinogenic properties. Further supportive of this hypothesis, cervical carcinogenesis in mice expressing a mutant form of HPV-16 E7, E7^{CVQ}, which fails to inactivate p21^{Cip1}, was significantly reduced compared with that in K14E7^{WT} mice expressing wild-type HPV-16 E7. However, K14E7^{CVQ} mice still displayed heightened levels of cervical carcinogenesis compared with that in nontransgenic mice, indicating that activities of E7 besides its capacity to inactivate p21^{Cip1} also contribute to cervical carcinogenesis. Taken together, we conclude that p21^{Cip1} functions as a tumor suppressor in cervical carcinogenesis and that p21^{Cip1} inactivation by HPV-16 E7 partially contributes to the contribution of E7 to cervical carcinogenesis.

10. Computational Identification of a p38^{SAPK}-Regulated Transcription Factor Network Required for Tumor Cell Quiescence, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5664-5672

ABSTRACT: The stress-activated kinase p38 plays key roles in tumor suppression and induction of tumor cell dormancy. However, the mechanisms behind these functions remain poorly understood. Using computational tools, we identified a transcription factor (TF) network regulated by p38 α/β and required for human squamous carcinoma cell quiescence in vivo. We found that p38 transcriptionally regulates a core network of 46 genes that includes 16 TFs. Activation of p38 induced the expression of the TFs p53 and BHLHB3, while inhibiting c-Jun and FoxM1 expression. Furthermore, induction of p53 by p38 was dependent on c-Jun down-regulation. Accordingly, RNAi down-regulation of BHLHB3 or p53 interrupted tumor cell quiescence, while down-regulation of c-Jun or FoxM1 or overexpression of BHLHB3 in malignant cells mimicked the onset of quiescence. Our results identify components of the regulatory mechanisms driving p38-induced cancer cell quiescence. These may regulate dormancy of residual disease that usually precedes the onset of metastasis in many cancers.

11. Overexpression of *DPAGT1* Leads to Aberrant N-Glycosylation of E-Cadherin and Cellular Discohesion in Oral Cancer, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5673-5680

ABSTRACT: Cancer cells are frequently characterized by aberrant increases in protein N-glycosylation and by disruption of E-cadherin-mediated adherens junctions. The relationship between altered N-glycosylation and loss of E-cadherin adhesion in cancer, however, remains unclear. Previously, we reported that complex N-glycans on the extracellular domains of E-cadherin inhibited the formation of mature adherens junctions. Here, we examined whether dysregulated N-glycosylation was one of the underlying causes for cellular discohesion in oral cancer. We show that dense cultures of human salivary epidermoid carcinoma A253 cells exhibited elevated expression of *DPAGT1*, the gene that initiates protein N-glycosylation. Overexpression of *DPAGT1* correlated with the production of E-cadherin-bearing complex N-glycans in nascent adherens junctions. Partial inhibition of *DPAGT1* with small interfering RNA reduced the complex N-glycans of E-cadherin and increased the abundance of α -catenin and stabilizing proteins in adherens junctions. This was associated with the assembly of functional tight junctions. The inverse relationship between *DPAGT1* expression and intercellular adhesion was a feature of oral squamous cell carcinoma. Oral squamous cell carcinomas displayed overexpression of *DPAGT1* that correlated with diminished localization of E-cadherin and α -catenin at the sites of adherens junctions. Our studies show for the first time that *DPAGT1* is an upstream regulator of E-cadherin N-glycosylation status and adherens junction composition and suggest that dysregulation of *DPAGT1* causes disturbances in intercellular adhesion in oral cancer.

12. Kruppel-Associated Box Domain-Associated Protein-1 as a Latency Regulator for Kaposi's Sarcoma-Associated Herpesvirus and Its Modulation by the Viral Protein Kinase, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5681-5689

ABSTRACT: Kaposi's sarcoma-associated herpesvirus (KSHV) has been linked to the development of Kaposi's sarcoma, a major AIDS-associated malignancy, and to hematologic malignancies, including primary effusion lymphoma and multicentric Castlemann's disease. Like other herpesviruses, KSHV is capable of both latent and lytic replication. Understanding the molecular details associated with this transition from latency to lytic replication is key to controlling virus spread and can affect the development of intervention strategies. Here, we report that Kruppel-associated box domain-associated protein-1 (KAP-1)/transcriptional intermediary factor 1 β , a cellular transcriptional repressor that controls chromosomal remodeling, participates in the process of switching viral latency to lytic replication. Knockdown of KAP-1 by small interfering RNA leads to KSHV reactivation mediated by K-Rta, a key transcriptional regulator. In cells harboring latent KSHV, KAP-1 was associated with the majority of viral lytic-gene promoters. K-Rta overexpression induced the viral lytic cycle with concomitant reduction of KAP-1 binding to viral promoters. Association of KAP-1 with heterochromatin was modulated by both sumoylation and phosphorylation. During lytic replication of KSHV, KAP-1 was phosphorylated at Ser⁸²⁴. Several lines of evidence directly linked the viral protein kinase to this post-translational modification. Additional studies showed that this phosphorylation of KAP-1 produced a decrease in its sumoylation, consequently decreasing the ability of KAP-1 to condense chromatin on viral promoters. In summary, the cellular transcriptional repressor KAP-1 plays a role in regulating KSHV latency, and viral protein kinase modulates the chromatin remodeling function of this repressor.

13. The Serine Protease Inhibitor Protease Nexin-1 Controls Mammary Cancer Metastasis through LRP-1-Mediated MMP-9 Expression, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5690-5698

ABSTRACT: Through their ability to degrade the extracellular matrix, proteases mediate cancer cell invasion and metastasis. Paradoxically, some serine protease inhibitors (serpins) are often overexpressed in human tumors. Using computational analysis, we found that the RNA level of protease nexin-1 (PN-1), a serpin that blocks numerous proteases activity, is significantly elevated in estrogen receptor- α -negative and in high-grade breast cancer. The in silico approach was complemented by mechanistic studies on two mammary cancer cell lines, the PN-1-negative 168FARN cells and the PN-1-positive 4T1 cells, both of which form primary mammary tumors, but only 4T1 tumors are able to metastasize to the lungs. We show that treatment of 168FARN cells with PN-1 stimulates extracellular signal-regulated kinase activation via low-density lipoprotein receptor-related protein-1 (LRP-1) binding, resulting in increased matrix metalloproteinase (MMP)-9 RNA, protein, and secreted activity. PN-1-silenced 4T1 cells express low MMP-9 levels. Moreover, injection of PN-1-silenced cells into mice did not affect 4T1 primary mammary tumor outgrowth; however, the tumors had impaired metastatic potential, which could be restored by reexpressing soluble MMP-9 in the PN-1-silenced 4T1 cells. Thus, using mammary tumor models, we describe a novel pathway whereby the serpin PN-1 by binding LRP-1 stimulates extracellular signal-regulated kinase signaling, MMP-9 expression, and metastatic spread of mammary tumors. Importantly, an analysis of 126 breast cancer patients revealed that those whose breast tumors had elevated PN-1 levels had a significantly higher probability to develop lung metastasis, but not metastasis to other sites, on relapse. These results suggest that PN-1 might become a prognostic marker in breast cancer.

14. Suppression of Nonhomologous End Joining Repair by Overexpression of HMGA2, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5699-5706

ABSTRACT: Understanding the molecular details associated with aberrant high mobility group A2 (HMGA2) gene expression is key to establishing the mechanism(s) underlying its oncogenic potential and effect on the development of therapeutic strategies. Here, we report the involvement of HMGA2 in impairing DNA-dependent protein kinase (DNA-PK) during the nonhomologous end joining (NHEJ) process. We showed that HMGA2-expressing cells displayed deficiency in overall and precise DNA end-joining repair and accumulated more endogenous DNA damage. Proper and timely activation of DNA-PK, consisting of Ku70, Ku80, and DNA-PKcs subunits, is essential for the repair of DNA double strand breaks (DSB) generated endogenously or by exposure to genotoxins. In cells overexpressing HMGA2, accumulation of histone 2A variant X phosphorylation at Ser-139 (γ -H2AX) was associated with hyperphosphorylation of DNA-PKcs at Thr-2609 and Ser-2056 before and after the induction of DSBs. Also, the steady-state complex of Ku and DNA ends was altered by HMGA2. Microirradiation and real-time imaging in living cells revealed that HMGA2 delayed the release of DNA-PKcs from DSB sites, similar to observations found in DNA-PKcs mutants. Moreover, HMGA2 alone was sufficient to induce chromosomal aberrations, a hallmark of deficiency in NHEJ-mediated DNA repair. In summary, a novel role for HMGA2 to interfere with NHEJ processes was uncovered, implicating HMGA2 in the promotion of genome instability and tumorigenesis.

15. MUC1, a New Hypoxia Inducible Factor Target Gene, Is an Actor in Clear Renal Cell Carcinoma Tumor Progression, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5707-5715

ABSTRACT: The hypoxia inducible factor (HIF) signaling pathway is known as the main renal carcinogenetic pathway. MUC1, an O-glycoprotein membrane-bound mucin, is overexpressed in clear renal cell carcinomas (cRCC) with correlation to two major prognostic factors: tumor-node-metastasis stage and nuclear F \ddot{u} rhman grade. We questioned whether there is a direct link between the HIF pathway and MUC1 overexpression in renal tumors. Interestingly, we observed concomitant increase of HIF-1 α and MUC1 in metastatic cRCC group versus nonmetastatic cRCC group. Using different renal cell models and small interfering RNA assays targeting either HIF-1 α or YC-1, a HIF-1 pharmacologic inhibitor, we showed induction of MUC1 expression under hypoxia by a HIF-dependent mechanism. Chromatin immunoprecipitation assay showed a direct binding of HIF-1 α at the MUC1 promoter. In addition, combined site-directed mutagenesis and gel shift assay allowed the identification of two functional putative hypoxia responsive elements at -1488/-1485 and at -1510/-1507 in the promoter. Using a rat kidney model of ischemia/reperfusion, we confirmed in vivo that clamping renal pedicle for 1 hour followed by 2 hours of reperfusion induced increased MUC1 expression. Furthermore, MUC1 knockdown induced significant reduction of invasive and migration properties of renal cancer cells under hypoxia. Altogether, these results show that MUC1 is directly regulated by HIF-1 α and affects the invasive and migration properties of renal cancer cells. Thus, MUC1 could serve as a potential therapeutic target in cRCC.

16. Depletion of Embryonic Stem Cell Signature by Histone Deacetylase Inhibitor in NCCIT Cells: Involvement of Nanog Suppression, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5716-5725

ABSTRACT: The embryonic stem cell-like gene expression signature has been shown to be associated with poorly differentiated aggressive human tumors and has attracted great attention as a potential target for future cancer therapies. Here, we investigate the potential of the embryonic stem cell signature as molecular target for the therapy and the strategy to suppress the embryonic stem cell signature. The core stemness gene Nanog is abnormally overexpressed in human embryonic carcinoma NCCIT cells showing gene expression profiles similar to embryonic stem cells. Down-regulation of the gene by either small interfering RNAs targeting Nanog or histone deacetylase inhibitor apicidin causes reversion of expression pattern of embryonic stem cell signature including Oct4, Sox2, and their target genes, leading to cell cycle arrest, inhibition of colony formation in soft agar, and induction of differentiation into all three germ layers. These effects are antagonized by reintroduction of Nanog. Interestingly, embryonic carcinoma cells (NCCIT, NTERA2, and P19) exhibit a higher sensitivity to apicidin in down-regulation of Nanog compared with embryonic stem cells. Furthermore, the down-regulation of Nanog expression by apicidin is mediated by a coordinated change in recruitment of epigenetic modulators and transcription factors to the promoter region. These findings indicate that overexpression of stemness gene Nanog in NCCIT cells is associated with maintaining stem cell-like phenotype and suggest that targeting Nanog might be an approach for improved therapy of poorly differentiated tumors.

17. Prostaglandin F_{2α}-F-Prostanoid Receptor Signaling Promotes Neutrophil Chemotaxis via Chemokine (C-X-C Motif) Ligand 1 in Endometrial Adenocarcinoma, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5726-5733

ABSTRACT: The prostaglandin F_{2α} (PGF_{2α}) receptor (FP) is elevated in endometrial adenocarcinoma. This study found that PGF_{2α} signaling via FP regulates expression of chemokine (C-X-C motif) ligand 1 (CXCL1) in endometrial adenocarcinoma cells. Expression of CXCL1 and its receptor, CXCR2, are elevated in cancer tissue compared with normal endometrium and localized to glandular epithelium, endothelium, and stroma. Treatment of Ishikawa cells stably transfected with the FP receptor (FPS cells) with 100 nmol/L PGF_{2α} increased CXCL1 promoter activity, mRNA, and protein expression, and these effects were abolished by cotreatment of cells with FP antagonist or chemical inhibitors of Gq, epidermal growth factor receptor, and extracellular signal-regulated kinase. Similarly, CXCL1 was elevated in response to 100 nmol/L PGF_{2α} in endometrial adenocarcinoma explant tissue. CXCL1 is a potent neutrophil chemoattractant. The expression of CXCR2 colocalized to neutrophils in endometrial adenocarcinoma and increased neutrophils were present in endometrial adenocarcinoma compared with normal endometrium. Conditioned media from PGF_{2α}-treated FPS cells stimulated neutrophil chemotaxis, which could be abolished by CXCL1 protein immunoneutralization of the conditioned media or antagonism of CXCR2. Finally, xenograft tumors in nude mice arising from inoculation with FPS cells showed increased neutrophil infiltration compared with tumors arising from wild-type cells or following treatment of mice bearing FPS tumors with CXCL1-neutralizing antibody. In conclusion, our results show a novel PGF_{2α}-FP pathway that may regulate the inflammatory microenvironment in endometrial adenocarcinoma via neutrophil chemotaxis.

18. Epstein-Barr Virus Latent Membrane Protein-1 Effects on Junctional Plakoglobin and Induction of a Cadherin Switch, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5734-5742

ABSTRACT: Latent membrane protein-1 (LMP1) is considered the major oncoprotein of Epstein-Barr virus and is frequently expressed in nasopharyngeal carcinoma (NPC). LMP1 promotes growth and migration of epithelial cells, and the loss of plakoglobin has been identified as a contributing factor to LMP1-induced migration. Plakoglobin is a junctional protein that can also serve as a transcription factor in Tcf/Lef signaling. To determine the effects of LMP1 on the molecular and functional properties of plakoglobin, LMP1 was overexpressed in the NPC cell line C666-1. LMP1 did not affect plakoglobin stability but did decrease plakoglobin transcription. The resultant decreased levels of nuclear plakoglobin did not affect Tcf/Lef activity or the amount of plakoglobin bound to Tcf4. Although LMP1 induced and stabilized β-catenin, a protein with common binding partners to plakoglobin, the loss of plakoglobin did not affect its association with Tcf4. However, LMP1 did induce a cadherin switch from E- to N-cadherin, a process involved in cancer progression, and enhanced the association of junctional β-catenin with N-cadherin. LMP1 decreased overall levels of junctional plakoglobin but the remaining junctional plakoglobin was found associated with the induced N-cadherin. This increased association of junctional plakoglobin with N-cadherin was a distinguishing feature of LMP1-expressing cells that have reduced migration due to restoration of plakoglobin. Low levels of plakoglobin were also detected in human NPC tissues. These findings reveal that the effects of LMP1 on junctional plakoglobin and the initiation of a cadherin switch likely contribute to metastasis of NPC.

19. Breast Cancer Migration and Invasion Depend on Proteasome Degradation of Regulator of G-Protein Signaling 4, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5743-5751

ABSTRACT: Aberrant signaling through G-protein coupled receptors promotes metastasis, the major cause of breast cancer death. We identified regulator of G-protein signaling 4 (RGS4) as a novel suppressor of breast cancer migration and invasion, important steps of metastatic cascades. By blocking signals initiated through G_i-coupled receptors, such as protease-activated receptor 1 and CXC chemokine receptor 4, RGS4 disrupted Rac1-dependent lamellipodia formation, a key step involved in cancer migration and invasion. RGS4 has GTPase-activating protein (GAP) activity, which inhibits G-protein coupled receptor signaling by deactivating G-proteins. An RGS4 GAP-deficient mutant failed to inhibit migration and invasion of breast cancer cells in both in vitro assays and a mouse xenograft model. Interestingly, both established breast cancer cell lines and human breast cancer specimens showed that the highest levels of RGS4 protein were expressed in normal breast epithelia and that RGS4 down-regulation by proteasome degradation is an index of breast cancer invasiveness. Proteasome blockade increased endogenous RGS4 protein to levels that markedly inhibit breast cancer cell migration and invasion, which was reversed by an RGS4-targeted short hairpin RNA. Our findings point to the existence of a mechanism for posttranslational regulation of RGS4 function, which may have important implications for the acquisition of a metastatic phenotype by breast cancer cells. Preventing degradation of RGS4 protein should attenuate aberrant signal inputs from multiple G_i-coupled receptors, thereby retarding the spread of breast cancer cells and making them targets for surgery, radiation, and immune treatment.

20. The Cell Fate Determination Factor DACH1 Is Expressed in Estrogen Receptor- α -Positive Breast Cancer and Represses Estrogen Receptor- α Signaling, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5752-5760

ABSTRACT: The Dachshund (dac) gene, initially cloned as a dominant inhibitor of the Drosophila hyperactive EGFR mutant ellipse, encodes a key component of the cell fate determination pathway involved in Drosophila eye development. Analysis of more than 2,200 breast cancer samples showed improved survival by some 40 months in patients whose tumors expressed DACH1. Herein, DACH1 and estrogen receptor- α (ER α) expressions were inversely correlated in human breast cancer. DACH1 bound and inhibited ER α function. Nuclear DACH1 expression inhibited estradiol (E₂)-induced DNA synthesis and cellular proliferation. DACH1 bound ER α in immunoprecipitation-Western blotting, associated with ER α in chromatin immunoprecipitation, and inhibited ER α transcriptional activity, requiring a conserved DS domain. Proteomic analysis identified proline, glutamic acid, and leucine rich protein 1 (PELP1) as a DACH1-binding protein. The DACH1 COOH terminus was required for binding to PELP1. DACH1 inhibited induction of ER α signaling. E₂ recruited ER α and disengaged corepressors from DACH1 at an endogenous ER response element, allowing PELP1 to serve as an ER α coactivator. DACH1 expression, which is lost in poor prognosis human breast cancer, functions as an endogenous inhibitor of ER α function.

21. MiR-122/Cyclin G1 Interaction Modulates p53 Activity and Affects Doxorubicin Sensitivity of Human Hepatocarcinoma Cells, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5761-5767

ABSTRACT: The identification of target genes is a key step for assessing the role of aberrantly expressed microRNAs (miRNA) in human cancer and for the further development of miRNA-based gene therapy. MiR-122 is a liver-specific miRNA accounting for 70% of the total miRNA population. Its down-regulation is a common feature of both human and mouse hepatocellular carcinoma (HCC). We have previously shown that miR-122 can regulate the expression of cyclin G1, whose high levels have been reported in several human cancers. We evaluated the role of miR-122 and cyclin G1 expression in hepatocarcinogenesis and in response to treatment with doxorubicin and their relevance on survival and time to recurrence (TTR) of HCC patients. We proved that, by modulating cyclin G1, miR-122 influences p53 protein stability and transcriptional activity and reduces invasion capability of HCC-derived cell lines. In addition, in a therapeutic perspective, we assayed the effects of a restored miR-122 expression in triggering doxorubicin-induced apoptosis and we proved that miR-122, as well as cyclin G1 silencing, increases sensitivity to doxorubicin challenge. In patients resected for HCC, lower miR-122 levels were associated with a shorter TTR, whereas higher cyclin G1 expression was related to a lower survival, suggesting that miR-122 might represent an effective molecular target for HCC. Our findings establish a basis toward the development of combined chemo- and miRNA-based therapy for HCC treatment.

22. Long-lived Min Mice Develop Advanced Intestinal Cancers through a Genetically Conservative Pathway, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5768-5775

ABSTRACT: C57BL/6J mice carrying the Min allele of Adenomatous polyposis coli (Apc) develop numerous adenomas along the entire length of the intestine and consequently die at an early age. This short lifespan would prevent the accumulation of somatic genetic mutations or epigenetic alterations necessary for tumor progression. To overcome this limitation, we generated F₁ Apc^{Min/+} hybrids by crossing C57BR/cdcJ and SWR/J females to C57BL/6J Apc^{Min/+} males. These hybrids developed few intestinal tumors and often lived longer than 1 year. Many of the tumors (24-87%) were invasive adenocarcinomas, in which neoplastic tissue penetrated through the muscle wall into the mesentery. In a few cases (3%), lesions metastasized by extension to regional lymph nodes. The development of these familial cancers does not require chromosomal gains or losses, a high level of microsatellite instability, or the presence of Helicobacter. To test whether genetic instability might accelerate tumor progression, we generated Apc^{Min/+} mice homozygous for the hypomorphic allele of the Nijmegen breakage syndrome gene (Nbs1^{ΔB}) and also treated Apc^{Min/+} mice with a strong somatic mutagen. These imposed genetic instabilities did not reduce the time required for cancers to form nor increase the percentage of cancers nor drive progression to the point of distant metastasis. In summary, we have found that the Apc^{Min/+} mouse model for familial intestinal cancer can develop frequent invasive cancers in the absence of overt genomic instability. Possible factors that promote invasion include age-dependent epigenetic changes, conservative somatic recombination, or direct effects of alleles in the F₁ hybrid genetic background.

23. MicroRNA Classifiers for Predicting Prognosis of Squamous Cell Lung Cancer, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5776-5783

ABSTRACT: Non-small cell lung cancer (NSCLC), which is comprised mainly of adenocarcinoma and squamous cell carcinoma (SCC), is the cause of 80% of all lung cancer deaths in the United States. NSCLC is also associated with a high rate of relapse after clinical treatment and, therefore, requires robust prognostic markers to better manage therapy options. The aim of this study was to identify microRNA (miRNA) expression profiles in SCC of the lung that would better predict prognosis. Total RNA from 61 SCC samples and 10 matched normal lung samples was processed for small RNA species and profiled on MirVana miRNA Bioarrays (version 2, Ambion). We identified 15 miRNAs that were differentially expressed between normal lung and SCC, including members of the miR-17-92 cluster and its paralogues. We also identified miRNAs, including miR-155 and let-7, which had previously been shown to have prognostic value in adenocarcinoma. Based on cross-fold validation analyses, miR-146b alone was found to have the strongest prediction accuracy for stratifying prognostic groups at ~78%. The miRNA signatures were superior in predicting overall survival than a previously described 50-gene prognostic signature. Whereas there was no overlap between the mRNAs targeted by the prognostic miRNAs and the 50-gene expression signature, there was a significant overlap in the corresponding biological pathways, including fibroblast growth factor and interleukin-6 signaling. Our data indicate that miRNAs may have greater clinical utility in predicting the prognosis of patients with squamous cell lung carcinomas than mRNA-based signatures.

24. Bone Morphogenetic Proteins 2 and 5 Are Down-regulated in Adrenocortical Carcinoma and Modulate Adrenal Cell Proliferation and Steroidogenesis, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5784-5792

ABSTRACT: Bone morphogenetic proteins (BMP) have been shown to affect tumorigenesis in a variety of tumors. Quantitative PCR analysis revealed down-regulation of BMP2 and BMP5 in tissue samples from adrenocortical carcinoma and adrenocortical tumor cell lines compared with normal adrenal glands. Integrity of BMP-dependent pathways in these cell lines could be shown by activation of the Smad1/5/8 pathway with subsequent increase of ID protein expression upon incubation with BMP2 or BMP5. On a functional level, BMP treatment resulted in inhibition of cell proliferation and viability in a dose- and time-dependent manner. This growth inhibitory effect was associated with BMP-dependent reduction of AKT phosphorylation under baseline conditions and under insulin-like growth factor costimulation. Furthermore, steroidogenic function, including melanocortin-2 receptor and steroidogenic enzyme expressions, was profoundly reduced. In vitro demethylation treatment and overexpression of GATA6 resulted in reactivation of BMP-dependent pathways with concomitant modulation of steroidogenesis. Taken together, we show that loss of expression of members of the BMP family of ligands is a common finding in adrenocortical tumors and we provide evidence that BMP-dependent pathways are likely to be involved in the modulation of the malignant and functional phenotype of adrenocortical cancer cells.

25. Positive Feedback Activation of Estrogen Receptors by the CXCL12-CXCR4 Pathway, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5793-5800

ABSTRACT: Induction of estrogen-regulated gene transcription by estrogen receptors ER α and ER β plays an important role in breast cancer development and growth. High expression of the chemokine receptor CXCR4 and its ligand CXCL12/stromal cell-derived factor 1 (SDF-1) has also been correlated with aggressive breast tumor phenotypes. Here, we describe a positive regulatory loop between the CXCR4/SDF-1 signaling pathway and ER transcriptional competence in human breast cancer cells. Treatment of breast carcinoma MCF-7 cells with SDF-1 increased ER transcriptional activity and expression of ER target genes, including SDF-1 itself. These effects were blocked by the antiestrogen ICI-182780 and by CXCR4 silencing and, conversely, estrogen-induced gene expression and growth of MCF-7 cells were impaired on CXCR4 inhibition. Both ER α and ER β were activated by SDF-1 in the presence of CXCR4 and by overexpression of a constitutively active CXCR4, indicating that CXCR4 signals to both receptors. In particular, ER β was able to translate the effects of SDF-1 on its own expression, as well as enhance activator protein 1 (AP-1) containing genes cyclin D1 and c-Myc in the presence of tamoxifen. This correlated with an increased ER β occupancy of responsive promoters at both estrogen-responsive and AP-1 elements. Ser-87, a conserved mitogen-activated protein kinase site in ER β , was highly phosphorylated by SDF-1, revealing an essential role of the AF-1 domain in response to CXCR4 activation. These results identify a complete autocrine loop between the CXCR4/SDF-1 and ER α /ER β signaling pathways that dictates ER-dependent gene expression and growth of breast cancer cells.

26. Modification of Ovarian Cancer Risk by *BRCA1/2*-Interacting Genes in a Multicenter Cohort of *BRCA1/2* Mutation Carriers, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5801-5810

ABSTRACT: Inherited *BRCA1/2* mutations confer elevated ovarian cancer risk. Knowledge of factors that can improve ovarian cancer risk assessment in *BRCA1/2* mutation carriers is important because no effective early detection for ovarian cancers exists. A cohort of 1,575 *BRCA1* and 856 *BRCA2* mutation carriers was used to evaluate haplotypes at *ATM*, *BARD1*, *BRIP1*, *CTIP*, *MRE11*, *NBS1*, *RAD50*, *RAD51*, and *TOPBP1* in ovarian cancer risk. In *BRCA1* carriers, no associations were observed with *ATM*, *BARD1*, *CTIP*, *RAD50*, *RAD51*, or *TOPBP1*. At *BRIP1*, an association was observed for one haplotype with a multiple testing corrected P (P_{corr}) = 0.012, although no individual haplotype was significant. At *MRE11*, statistically significant associations were observed for one haplotype (P_{corr} = 0.007). At *NBS1*, we observed a P_{corr} = 0.024 for haplotypes. In *BRCA2* carriers, no associations were observed with *CTIP*, *NBS1*, *RAD50*, or *TOPBP1*. Rare haplotypes at *ATM* (P_{corr} = 0.044) and *BARD1* (P_{corr} = 0.012) were associated with ovarian cancer risk. At *BRIP1*, two common haplotypes were significantly associated with ovarian cancer risk (P_{corr} = 0.011). At *MRE11*, we observed a significant haplotype association (P_{corr} = 0.012), and at *RAD51*, one common haplotype was significantly associated with ovarian cancer risk (P_{corr} = 0.026). Variants in genes that interact biologically with *BRCA1* and/or *BRCA2* may be associated with modified ovarian cancer risk in women who carry *BRCA1/2* mutations.

27. Analysis of the Human Cancer Glycome Identifies a Novel Group of Tumor-Associated *N*-Acetylglucosamine Glycan Antigens, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5811-5819

ABSTRACT: The cell surface is covered by a dense layer of protein- and lipid-linked glycans. Although it has been known that distinct glycan structures are associated with cancer, the whole spectrum of cancer-associated glycans has remained undiscovered. In the present study, we analyzed the protein-linked cancer glycome by matrix-assisted laser desorption/ionization time-of-flight mass spectrometric glycan profiling of cancer patient tissue samples. In lung cancer, we detected accumulation of a novel group of tumor-associated glycans. These protein-linked glycans carried abnormal nonreducing terminal β -*N*-acetyl-D-glucosamine (GlcNAc) residues. A similar phenomenon was also detected in structural analyses of tumor-derived glycosphingolipids. This showed that glycan biosynthesis may dramatically change in cancer and that direct glycome analysis can detect the resulting marker glycans. Based on the structural knowledge, we further devised a covalent labeling technique for the detection of GlcNAc-expressing tumors with a specific transferase enzyme. In normal tissues, terminal GlcNAc antigens are capped by galactosylation. Similarly to common cancer-associated glycan antigens T, Tn, and sialyl-Tn, the newly discovered GlcNAc antigens result from incomplete glycosylation. In conclusion, the identified terminal GlcNAc glycans should be recognized as a novel class of tumor markers.

28. Epithelial to Mesenchymal Transition Contributes to Drug Resistance in Pancreatic Cancer, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5820-5828

ABSTRACT: A better understanding of drug resistance mechanisms is required to improve outcomes in patients with pancreatic cancer. Here, we characterized patterns of sensitivity and resistance to three conventional chemotherapeutic agents with divergent mechanisms of action [gemcitabine, 5-fluorouracil (5-FU), and cisplatin] in pancreatic cancer cells. Four (L3.6pl, BxPC-3, CFPAC-1, and SU86.86) were sensitive and five (PANC-1, Hs766T, AsPC-1, MIAPaCa-2, and MPanc96) were resistant to all three agents based on GI_{50} (50% growth inhibition). Gene expression profiling and unsupervised hierarchical clustering revealed that the sensitive and resistant cells formed two distinct groups and differed in expression of specific genes, including several features of "epithelial to mesenchymal transition" (EMT). Interestingly, an inverse correlation between E-cadherin and its transcriptional suppressor, Zeb-1, was observed in the gene expression data and was confirmed by real-time PCR. Independent validation experiment using five new pancreatic cancer cell lines confirmed that an inverse correlation between E-cadherin and Zeb-1 correlated closely with resistance to gemcitabine, 5-FU, and cisplatin. Silencing Zeb-1 in the mesenchymal lines not only increased the expression of E-cadherin but also other epithelial markers, such as EVA1 and MAL2, and restored drug sensitivity. Importantly, immunohistochemical analysis of E-cadherin and Zeb-1 in primary tumors confirmed that expression of the two proteins was mutually exclusive (P = 0.012). Therefore, our results suggest that Zeb-1 and other regulators of EMT may maintain drug resistance in human pancreatic cancer cells, and therapeutic strategies to inhibit Zeb-1 and reverse EMT should be evaluated.

29. Preclinical Development of a Bifunctional Cancer Cell Homing, PKC ϵ Inhibitory Peptide for the Treatment of Head and Neck Cancer, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5829-5834

ABSTRACT: Head and neck squamous cell carcinoma (HNSCC) is the sixth most frequent cancer worldwide, comprising ~50% of all malignancies in some developing nations. Our recent work identified protein kinase C ϵ (PKC ϵ) as a critical and causative player in establishing an aggressive phenotype in HNSCC. In this study, we investigated the specificity and efficacy of HN1-PKC ϵ , a novel bifunctional cancer cell homing, PKC ϵ inhibitory peptide, as a treatment for HNSCC. HN1-PKC ϵ peptide was designed by merging two separate technologies and synthesized as a capped peptide with two functional modules, HN1 (cancer cell homing) and PKC ϵ (specific PKC ϵ inhibitory), connected by a novel linker module. HN1-PKC ϵ preferentially internalized into UMSCC1 and UMSCC36 cells, two HNSCC cell lines, in comparison with oral epithelial cells: 82.1% positive for UMSCC1 and 86.5% positive for UMSCC36 compared with 1.2% positive for oral epithelial cells. In addition, HN1-PKC ϵ penetrated HNSCC cells in a dose- and time-dependent manner. Consistent with these in vitro observations, systemic injection of HN1-PKC ϵ resulted in selective delivery of HN1-PKC ϵ into UMSCC1 xenografts in nude mice. HN1-PKC ϵ blocked the translocation of active PKC ϵ in UMSCC1 cells, confirming HN1-PKC ϵ as a PKC ϵ inhibitor. HN1-PKC ϵ inhibited cell invasion by $72 \pm 2\%$ ($P < 0.001$, $n = 12$) and cell motility by $56 \pm 2\%$ ($P < 0.001$, $n = 5$) in UMSCC1 cells. Moreover, in vivo bioluminescence imaging showed that HN1-PKC ϵ significantly ($83 \pm 1\%$ inhibition; $P < 0.02$) retards the growth of UMSCC1 xenografts in nude mice. Our work indicates that the bifunctional HN1-PKC ϵ inhibitory peptide represents a promising novel therapeutic strategy for HNSCC.

30. Antimyeloma Activity of the Orally Bioavailable Dual Phosphatidylinositol 3-Kinase/Mammalian Target of Rapamycin Inhibitor NVP-BEZ235, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5835-5842

ABSTRACT: The phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway mediates proliferation, survival, and drug resistance in multiple myeloma (MM) cells. Here, we tested the anti-MM activity of NVP-BEZ235 (BEZ235), which inhibits PI3K/Akt/mTOR signaling at the levels of PI3K and mTOR. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric survival assays showed that MM cell lines exhibited dose- and time-dependent decreased viability after exposure to BEZ235 (IC_{50} , 25–800 nmol/L for 48 hours). MM cells highly sensitive (IC_{50} , <25 nmol/L) to BEZ235 (e.g., MM.1S, MM.1R, Dox40, and KMS-12-PE) included both lines sensitive and resistant to conventional (dexamethasone, cytotoxic chemotherapeutics) agents. Pharmacologically relevant BEZ235 concentrations (25–400 nmol/L) induced rapid commitment to and induction of MM.1S and OPM-2 cell death. Furthermore, normal donor peripheral blood mononuclear cells were less sensitive (IC_{50} , >800 nmol/L) than the majority of MM cell lines tested, suggesting a favorable therapeutic index. In addition, BEZ235 was able to target MM cells in the presence of exogenous interleukin-6, insulin-like growth factor-1, stromal cells, or osteoclasts, which are known to protect against various anti-MM agents. Molecular profiling revealed that BEZ235 treatment decreased the amplitude of transcriptional signatures previously associated with myc, ribosome, and proteasome function, as well as high-risk MM and undifferentiated human embryonic stem cells. In vivo xenograft studies revealed significant reduction in tumor burden ($P = 0.011$) and survival ($P = 0.028$) in BEZ235-treated human MM tumor-bearing mice. Combinations of BEZ235 with conventional (e.g., dexamethasone and doxorubicin) or novel (e.g., bortezomib) anti-MM agents showed lack of antagonism. These results indicate that BEZ235 merits clinical testing, alone and in combination with other agents, in MM.

31. Targeted *In vivo* Imaging of Integrin $\alpha_v\beta_6$ with an Improved Radiotracer and Its Relevance in a Pancreatic Tumor Model, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5843-5850

ABSTRACT: The cell surface receptor $\alpha_v\beta_6$ is epithelial specific, and its expression is tightly regulated; it is low or undetectable in adult tissues but has been shown to be increased in many different cancers, including pancreatic, cervical, lung, and colon cancers. Studies have described $\alpha_v\beta_6$ as a prognostic biomarker linked to poor survival. We have recently shown the feasibility of imaging $\alpha_v\beta_6$ in vivo by positron emission tomography (PET) using the peptide [^{18}F]FBA-A20FMDV2. Here, we describe improved $\alpha_v\beta_6$ imaging agents and test their efficacy in a mouse model with endogenous $\alpha_v\beta_6$ expression. The modified compounds maintained high affinity for $\alpha_v\beta_6$ and >1,000-fold selectivity over related integrins (by ELISA) and showed significantly improved $\alpha_v\beta_6$ -dependent binding in cell-based assays (>60% binding versus <10% for [^{18}F]FBA-A20FMDV2). In vivo studies using either a melanoma cell line (transduced $\alpha_v\beta_6$ expression) or the BxPC-3 human pancreatic carcinoma cell line (endogenous $\alpha_v\beta_6$ expression) revealed that the modified compounds showed significantly improved tumor retention. This, along with good clearance of nonspecifically bound activity, particularly for the new radiotracer [^{18}F]FBA-PEG₂₈-A20FMDV2, resulted in improved PET imaging. Tumor/pancreas and tumor/blood biodistribution ratios of >23:1 and >47:1, respectively, were achieved at 4 hours. Significantly, [^{18}F]FBA-PEG₂₈-A20FMDV2 was superior to 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) in imaging the BxPC-3 tumors. Pancreatic ductal adenocarcinoma is highly metastatic and current preoperative evaluation of resectability using noninvasive imaging has limited success, with most patients having metastases at time of surgery. The fact that these tumors express $\alpha_v\beta_6$ suggests that this probe has significant potential for the in vivo detection of this malignancy, thus having important implications for patient care and therapy.

32. Nimotuzumab, an Antitumor Antibody that Targets the Epidermal Growth Factor Receptor, Blocks Ligand Binding while Permitting the Active Receptor Conformation, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5851-5859

ABSTRACT: Overexpression of the epidermal growth factor (EGF) receptor (EGFR) in cancer cells correlates with tumor malignancy and poor prognosis for cancer patients. For this reason, the EGFR has become one of the main targets of anticancer therapies. Structural data obtained in the last few years have revealed the molecular mechanism for ligand-induced EGFR dimerization and subsequent signal transduction, and also how this signal is blocked by either monoclonal antibodies or small molecules. Nimotuzumab (also known as h-R3) is a humanized antibody that targets the EGFR and has been successful in the clinics. In this work, we report the crystal structure of the Fab fragment of Nimotuzumab, revealing some unique structural features in the heavy variable domain. Furthermore, competition assays show that Nimotuzumab binds to domain III of the extracellular region of the EGFR, within an area that overlaps with both the surface patch recognized by Cetuximab (another anti-EGFR antibody) and the binding site for EGF. A computer model of the Nimotuzumab-EGFR complex, constructed by docking and molecular dynamics simulations and supported by mutagenesis studies, unveils a novel mechanism of action, with Nimotuzumab blocking EGF binding while still allowing the receptor to adopt its active conformation, hence warranting a basal level of signaling.

33. High Efficacy of a *Listeria*-Based Vaccine against Metastatic Breast Cancer Reveals a Dual Mode of Action, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5860-5866

ABSTRACT: Most cancer vaccines induce CTL responses to tumor-associated antigens (TAA). Killing of tumor cells occurs through TAA-specific CTL-mediated cytolysis. Here, we show that one preventive followed by two therapeutic immunizations with an attenuated *Listeria monocytogenes* (LM)-based vaccine eradicates all metastases and almost the entire primary tumor in the syngeneic, aggressive mouse breast tumor model 4T1. We provide strong evidence that this is due to the combined result of direct kill by *Listeria* infecting the tumor cells and by CTL responses against *Listeria* antigens. We showed by electron microscopy that LM expressing truncated listeriolysin O (LLO) and amino acid fragments 311 to 660 of TAA Mage-b (LM-LLO-Mage-b₃₁₁₋₆₆₀) and the control strain LM-LLO infect tumor cells in vitro and in vivo. In vitro data indicate that tumor cell death occurs through activation of NADP⁺ oxidase and increased intracellular Ca²⁺ levels, both resulting in the production of high ROS levels. Because both LM-LLO and LM-LLO-Mage-b₃₁₁₋₆₆₀ showed equally strong efficacies in vivo, we concluded that LM-LLO was crucial and Mage-b was of less importance. We found strong CTL responses to LM-LLO in the spleen, and depletion of CD8 T cells in vivo resulted in significant tumor regrowth (52%) in LM-LLO-vaccinated mice, indicating that LM-LLO-specific CTL indeed partially contributed to tumor cell kill in vivo. This dual mode of action of a *Listeria*-based vaccine has not been described before and may provide new directions in the development of more effective vaccines against metastatic breast cancer.

34. Identification of Inhibitors of ABCG2 by a Bioluminescence Imaging–Based High-Throughput Assay, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5867-5875

ABSTRACT: ABCG2 is a member of the ATP-binding cassette (ABC) family of transporters, the overexpression of which is associated with tumor resistance to a variety of chemotherapeutic agents. Accordingly, combining ABCG2 inhibitor(s) with chemotherapy has the potential to improve treatment outcome. To search for clinically useful ABCG2 inhibitors, a bioluminescence imaging (BLI)–based assay was developed to allow high-throughput compound screening. This assay exploits our finding that D-luciferin, the substrate of firefly luciferase (fLuc), is a specific substrate of ABCG2, and ABCG2 inhibitors block the export of D-luciferin and enhance bioluminescence signal by increasing intracellular D-luciferin concentrations. HEK293 cells, engineered to express ABCG2 and fLuc, were used to screen the Hopkins Drug Library that includes drugs approved by the Food and Drug Administration (FDA) as well as drug candidates that have entered phase II clinical trials. Forty-seven compounds showed BLI enhancement, a measure of anti-ABCG2 activity, of ≥ 5 -fold, the majority of which were not previously known as ABCG2 inhibitors. The assay was validated by its identification of known ABCG2 inhibitors and by confirming previously unknown ABCG2 inhibitors using established in vitro assays (e.g., mitoxantrone resensitization and BODIPY-prazosin assays). Glafenine, a potent new inhibitor, also inhibited ABCG2 activity in vivo. The BLI-based assay is an efficient method to identify new inhibitors of ABCG2. As they were derived from a FDA-approved compound library, many of the inhibitors uncovered in this study are ready for clinical testing.

35. Cucurbitacin B Induces Apoptosis by Inhibition of the JAK/STAT Pathway and Potentiates Antiproliferative Effects of Gemcitabine on Pancreatic Cancer Cells, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5876-5884

ABSTRACT: Pancreatic cancer is an aggressive malignancy that is generally refractory to chemotherapy, thus posing experimental and clinical challenges. In this study, the antiproliferative effect of the triterpenoid compound cucurbitacin B was tested in vitro and in vivo against human pancreatic cancer cells. Dose-response studies showed that the drug inhibited 50% growth of seven pancreatic cancer cell lines at 10^{-7} mol/L, whereas clonogenic growth was significantly inhibited at 5×10^{-8} mol/L. Cucurbitacin B caused dose- and time-dependent G₂-M-phase arrest and apoptosis of pancreatic cancer cells. This was associated with inhibition of activated JAK2, STAT3, and STAT5, increased level of p21^{WAF1} even in cells with nonfunctional p53, and decrease of expression of cyclin A, cyclin B1, and Bcl-XL with subsequent activation of the caspase cascade. Interestingly, the combination of cucurbitacin B and gemcitabine synergistically potentiated the antiproliferative effects of gemcitabine on pancreatic cancer cells. Moreover, cucurbitacin B decreased the volume of pancreatic tumor xenografts in athymic nude mice by 69.2% ($P < 0.01$) compared with controls without noticeable drug toxicities. In vivo activation of JAK2/STAT3 was inhibited and expression of Bcl-XL was decreased, whereas caspase-3 and caspase-9 were up-regulated in tumors of drug-treated mice. In conclusion, we showed for the first time that cucurbitacin B has profound in vitro and in vivo antiproliferative effects against human pancreatic cancer cells, and the compound may potentiate the antiproliferative effect of the chemotherapeutic agent gemcitabine. Further clinical studies are necessary to confirm our findings in patients with pancreatic cancer.

36. Compartment-Specific Roles of ATP-Binding Cassette Transporters Define Differential Topotecan Distribution in Brain Parenchyma and Cerebrospinal Fluid, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5885-5892

ABSTRACT: Topotecan is a substrate of the ATP-binding cassette transporters P-glycoprotein (P-gp/MDR1) and breast cancer resistance protein (BCRP). To define the role of these transporters in topotecan penetration into the ventricular cerebrospinal fluid (vCSF) and brain parenchymal extracellular fluid (ECF) compartments, we performed intracerebral microdialysis on transporter-deficient mice after an intravenous dose of topotecan (4 mg/kg). vCSF penetration of unbound topotecan lactone was measured as the ratio of vCSF-to-plasma area under the concentration-time curves. The mean \pm SD ratios for wild-type, Mdr1a/b^{-/-}, Bcrp1^{-/-}, and Mdr1a/b^{-/-}Bcrp1^{-/-} mice were 3.07 ± 0.09 , 2.57 ± 0.17 , 1.63 ± 0.12 , and 0.86 ± 0.05 , respectively. In contrast, the ECF-to-plasma ratios for wild-type, Bcrp1^{-/-}, and Mdr1a/b^{-/-}Bcrp1^{-/-} mice were 0.36 ± 0.06 , 0.42 ± 0.06 , and 0.88 ± 0.07 . Topotecan lactone was below detectable limits in the ECF of Mdr1a/b^{-/-} mice. When gefitinib (200 mg/kg) was preadministered to inhibit Bcrp1 and P-gp, the vCSF-to-plasma ratio decreased to 1.29 ± 0.09 in wild-type mice and increased to 1.13 ± 0.13 in Mdr1a/b^{-/-}Bcrp1^{-/-} mice, whereas the ECF-to-plasma ratio increased to 0.74 ± 0.14 in wild-type and 1.07 ± 0.03 in Mdr1a/b^{-/-}Bcrp1^{-/-} mice. Preferential active transport of topotecan lactone over topotecan carboxylate was shown in vivo by vCSF lactone-to-carboxylate area under the curve ratios for wild-type, Mdr1a/b^{-/-}, Bcrp1^{-/-}, and Mdr1a/b^{-/-}Bcrp1^{-/-} mice of 5.69 ± 0.83 , 3.85 ± 0.64 , 3.61 ± 0.46 , and 0.78 ± 0.19 , respectively. Our results suggest that Bcrp1 and P-gp transport topotecan into vCSF and out of brain parenchyma through the blood-brain barrier. These findings may help to improve pharmacologic strategies to treat brain tumors.

37. Acetyl-11-Keto- β -Boswellic Acid Inhibits Prostate Tumor Growth by Suppressing Vascular Endothelial Growth Factor Receptor 2–Mediated Angiogenesis, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5893-5900

ABSTRACT: The role of angiogenesis in tumor growth and metastasis is well established. Identification of a small molecule that blocks tumor angiogenesis and is safe and affordable has been a challenge in drug development. In this study, we showed that acetyl-11-keto- β -boswellic acid (AKBA), an active component from an Ayurvedic medicinal plant (*Boswellia serrata*), could strongly inhibit tumor angiogenesis. AKBA suppressed tumor growth in the human prostate tumor xenograft mice treated daily (10 mg/kg AKBA) after solid tumors reached $\approx 100 \text{ mm}^3$ ($n = 5$). The inhibitory effect of AKBA on tumor growth was well correlated with suppression of angiogenesis. When examined for the molecular mechanism, we found that AKBA significantly inhibited blood vessel formation in the Matrigel plug assay in mice and effectively suppressed vascular endothelial growth factor (VEGF)–induced microvessel sprouting in rat aortic ring assay *ex vivo*. Furthermore, AKBA inhibited VEGF-induced cell proliferation, chemotactic motility, and the formation of capillary-like structures from primary cultured human umbilical vascular endothelial cells in a dose-dependent manner. Western blot analysis and *in vitro* kinase assay revealed that AKBA suppressed VEGF-induced phosphorylation of VEGF receptor 2 (VEGFR2) kinase (KDR/Flk-1) with IC_{50} of 1.68 $\mu\text{mol/L}$. Specifically, AKBA suppressed the downstream protein kinases of VEGFR2, including Src family kinase, focal adhesion kinase, extracellular signal-related kinase, AKT, mammalian target of rapamycin, and ribosomal protein S6 kinase. Our findings suggest that AKBA potentially inhibits human prostate tumor growth through inhibition of angiogenesis induced by VEGFR2 signaling pathways.

38. Secretion of Tumor-Specific Antigen by Myeloma Cells Is Required for Cancer Immunosurveillance by CD4^+ T Cells, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5901-5907

ABSTRACT: Tumor-specific CD4^+ T cells orchestrate the adaptive immune responses against cancer. We have previously shown that CD4^+ T cells recognize MHC class II–negative myeloma cells indirectly by collaborating with tumor-infiltrating macrophages. We, here, hypothesize that this critical step may be dependent on secretion of tumor-specific antigens by cancer cells. This was investigated using T-cell receptor–transgenic mice, in which CD4^+ T cells mediate rejection of syngeneic MOPC315 myeloma cells. We analyzed the immune response against myeloma cell variants, which either secrete or retain intracellularly a tumor-specific idiotype (Id) antigen. Our results reveal that CD4^+ T cells helped by macrophages are capable of detecting nonsecreted tumor antigens from MHC class II–negative cancer cells. However, Id secretion was required for successful myeloma immunosurveillance. Antigen secretion resulted in stronger priming of naive myeloma-specific CD4^+ T cells in tumor-draining lymph nodes. Secretion of antigen by at least some cancer cells within a tumor was shown to facilitate immunosurveillance. Treatment by local injection of purified tumor-specific antigen successfully enhanced immunity against nonsecreting myeloma cells. Collectively, the data indicate that antigen concentration within the tumor extracellular matrix must reach a certain threshold to allow successful cancer immunosurveillance by CD4^+ T cells.

39. Genomic Characterization of Esophageal Squamous Cell Carcinoma from a High-Risk Population in China, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5908-5917

ABSTRACT: Genomic instability plays an important role in most human cancers. To characterize genomic instability in esophageal squamous cell carcinoma (ESCC), we examined loss of heterozygosity (LOH), copy number (CN) loss, CN gain, and gene expression using the Affymetrix GeneChip Human Mapping 500K ($n = 30$ cases) and Human U133A ($n = 17$ cases) arrays in ESCC cases from a high-risk region of China. We found that genomic instability measures varied widely among cases and separated them into two groups: a high-frequency instability group (two-thirds of all cases with one or more instability category of $\geq 10\%$) and a low-frequency instability group (one-third of cases with instability of $< 10\%$). Genomic instability also varied widely across chromosomal arms, with the highest frequency of LOH on 9p (33% of informative single nucleotide polymorphisms), CN loss on 3p (33%), and CN gain on 3q (48%). Twenty-two LOH regions were identified: four on 9p, seven on 9q, four on 13q, two on 17p, and five on 17q. Three CN loss regions—3p12.3, 4p15.1, and 9p21.3—were detected. Twelve CN gain regions were found, including six on 3q, one on 7q, four on 8q, and one on 11q. One of the most gene-rich of these CN gain regions was 11q13.1-13.4, where 26 genes also had RNA expression data available. CN gain was significantly correlated with increased RNA expression in over 80% of these genes. Our findings show the potential utility of combining CN analysis and gene expression data to identify genes involved in esophageal carcinogenesis.

40. Progressive Tumor Formation in Mice with Conditional Deletion of TGF- β Signaling in Head and Neck Epithelia Is Associated with Activation of the PI3K/Akt Pathway, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5918-5926

ABSTRACT: The precise role of transforming growth factor (TGF)- β signaling in head and neck squamous cell carcinoma (SCC) is not yet fully understood. Here, we report generation of an inducible head- and neck-specific knockout mouse model by crossing TGF- β receptor I (Tgfr1) floxed mice with K14-CreER^{tam} mice. By applying tamoxifen to oral cavity of the mouse to induce Cre expression, we were able to conditionally delete Tgfr1 in the mouse head and neck epithelia. On tumor induction with 7,12-dimethylbenz(a)anthracene (DMBA), 45% of Tgfr1 conditional knockout (cKO) mice (n = 42) developed SCCs in the head and neck area starting from 16 weeks after treatment. However, no tumors were observed in the control littermates. A molecular analysis revealed an enhanced proliferation and loss of apoptosis in the basal layer of the head and neck epithelia of Tgfr1 cKO mice 4 weeks after tamoxifen and DMBA treatment. The most notable finding of our study is that the phosphoinositide 3-kinase (PI3K)/Akt pathway was activated in SCCs that developed in the Tgfr1 cKO mice on inactivation of TGF- β signaling through Smad2/3 and DMBA treatment. These observations suggest that activation of Smad-independent pathways may contribute cooperatively with inactivation of Smad-dependent pathways to promote head and neck carcinogenesis in these mice. Our results revealed the critical role of the TGF- β signaling pathway and its cross-talk with the PI3K/Akt pathway in suppressing head and neck carcinogenesis.

41. Protein Kinase C δ Activates RelA/p65 and Nuclear Factor- κ B Signaling in Response to Tumor Necrosis Factor- α , *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5927-5935

ABSTRACT: Nuclear factor- κ B (NF- κ B) is tightly modulated by I κ B kinases and I κ B α in the cytoplasm. On stimulation, NF- κ B translocates into the nucleus to initiate transcription; however, regulation of its transcriptional activity remains obscure. Here, we show that protein kinase C (PKC) δ controls the main subunit of NF- κ B, RelA/p65. On exposure to tumor necrosis factor- α (TNF- α), the expression of RelA/p65 target genes such as I κ B α , RelB, and p100/p52 is up-regulated in a PKC δ -dependent manner. The results also show that PKC δ is targeted to the nucleus and forms a complex with RelA/p65 following TNF- α exposure. Importantly, kinase activity of PKC δ is required for RelA/p65 transactivation. In concert with these results, PKC δ activates RelA/p65 for its occupancy to target-gene promoters, including I κ B α and p100/p52. Moreover, functional analyses show that inhibition of PKC δ is associated with substantial attenuation of NF- κ B activity in response to TNF- α . These findings provide evidence that PKC δ orchestrates RelA/p65 transactivation, a requisite for NF- κ B signaling pathway in the nucleus.

42. Xenoestrogen-Induced Epigenetic Repression of *microRNA-9-3* in Breast Epithelial Cells, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5936-5945

ABSTRACT: Early exposure to xenoestrogens may predispose to breast cancer risk later in adult life. It is likely that long-lived, self-regenerating epithelial progenitor cells are more susceptible to these exposure injuries over time and transmit the injured memory through epigenetic mechanisms to their differentiated progeny. Here, we used progenitor-containing mammospheres as an in vitro exposure model to study this epigenetic effect. Expression profiling identified that, relative to control cells, 9.1% of microRNAs (82 of 898 loci) were altered in epithelial progeny derived from mammospheres exposed to a synthetic estrogen, diethylstilbestrol. Repressive chromatin marks, trimethyl Lys27 of histone H3 (H3K27me3) and dimethyl Lys9 of histone H3 (H3K9me2), were found at a down-regulated locus, miR-9-3, in epithelial cells preexposed to diethylstilbestrol. This was accompanied by recruitment of DNA methyltransferase 1 that caused an aberrant increase in DNA methylation of its promoter CpG island in mammosphere-derived epithelial cells on diethylstilbestrol preexposure. Functional analyses suggest that miR-9-3 plays a role in the p53-related apoptotic pathway. Epigenetic silencing of this gene, therefore, reduces this cellular function and promotes the proliferation of breast cancer cells. Promoter hypermethylation of this microRNA may be a hallmark for early breast cancer development, and restoration of its expression by epigenetic and microRNA-based therapies is another viable option for future treatment of this disease.

43. Cytogenetic and cDNA Microarray Expression Analysis of MCF10 Human Breast Cancer Progression Cell Lines, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5946-5953

ABSTRACT: We used a combination of spectral karyotyping, array comparative genomic hybridization, and cDNA microarrays to gain insights into the structural and functional changes of the genome in the MCF10 human breast cancer progression model cell lines. Spectral karyotyping data showed several chromosomal aberrations and array comparative genomic hybridization analysis identified numerous genomic gains and losses that might be involved in the progression toward cancer. Analysis of the expression levels of genes located within these genomic regions revealed a lack of correlation between chromosomal gains and losses and corresponding up-regulation or down-regulation for the majority of the ~1,000 genes analyzed in this study. We conclude that other mechanisms of gene regulation that are not directly related to chromosomal gains and losses play a major role in breast cancer progression.

44. Activating Transcription Factor 2 and c-Jun–Mediated Induction of FoxP3 for Experimental Therapy of Mammary Tumor in the Mouse, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5954-5960

ABSTRACT: FOXP3 is inactivated in breast cancer cells by a number of mechanisms, including somatic mutations, deletion, and epigenetic silencing. Because the mutation and deletion are usually heterozygous in the cancer samples, it is of interest to determine whether the gene can be induced for the purpose of cancer therapy. Here, we report that anisomycin, a potent activator of activating transcription factor (ATF) 2, and c-Jun-NH₂-kinase, induces expression of FoxP3 in both normal and malignant mammary epithelial cells. The induction is mediated by ATF2 and c-Jun. Targeted mutation of ATF2 abrogates both constitutive and inducible expression of FoxP3 in normal epithelial cells. Both ATF2 and c-Jun interact with a novel enhancer in the intron 1 of the FoxP3 locus. Moreover, shRNA silencing of ATF2 and FoxP3 reveals an important role of ATF2-FoxP3 pathway in the anisomycin-induced apoptosis of breast cancer cells. A low dose of anisomycin was also remarkably effective in treating established mammary tumor in the mice. Our data showed that FoxP3 can be reactivated for cancer therapy.

45. Mice Heterozygous for Germ-line Mutations in Methylthioadenosine Phosphorylase (MTAP) Die Prematurely of T-Cell Lymphoma, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5961-5969

ABSTRACT: Large homozygous deletions of 9p21 that inactivate CDKN2A, ARF, and MTAP are common in a wide variety of human cancers. The role for CDKN2A and ARF in tumorigenesis is well established, but whether MTAP loss directly affects tumorigenesis is unclear. MTAP encodes the enzyme methylthioadenosine phosphorylase, a key enzyme in the methionine salvage pathway. To determine if loss of MTAP plays a functional role in tumorigenesis, we have created an MTAP-knockout mouse. Mice homozygous for a MTAP null allele (Mtap^{lacZ}) have an embryonic lethal phenotype dying around day 8 postconception. Mtap/Mtap^{lacZ} heterozygotes are born at Mendelian frequencies and appear indistinguishable from wild-type mice during the first year of life, but they tend to die prematurely with a median survival of 585 days. Autopsies on these animals reveal that they have greatly enlarged spleens, altered thymic histology, and lymphocytic infiltration of their livers, consistent with lymphoma. Immunohistochemical staining and fluorescence-activated cell sorting analysis indicate that these lymphomas are primarily T-cell in origin. Lymphoma-infiltrated tissues tend to have reduced levels of Mtap mRNA and MTAP protein in addition to unaltered levels of methyldeoxycytidine. These studies show that Mtap is a tumor suppressor gene independent of CDKN2A and ARF.

46. microRNA miR-196a-2 and Breast Cancer: A Genetic and Epigenetic Association Study and Functional Analysis, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5970-5977

ABSTRACT: Increasing evidence has suggested that microRNAs (miRNA) play an important role in tumorigenesis. As transcriptional regulators, altered miRNA expression may affect many cancer-related biological pathways, indicating that miRNAs can function as tumor suppressors and/or oncogenes. We first performed a genetic association analysis by screening genetic variants in 15 miRNA genes and detected that a common sequence variant in hsa-miR-196a-2 (rs11614913, C→T) was significantly associated with decreased breast cancer risk (for homozygous variant: odds ratio, 0.44; 95% confidence interval, 0.28-0.70). Hypermethylation of a CpG island upstream (-700 bp) of the miR-196a-2 precursor was also associated with reduced breast cancer risk (odds ratio, 0.35; 95% confidence interval, 0.15-0.81). By delivering expression vectors containing either wild-type or mutant precursors of miR-196a-2 into breast cancer cells, we showed that this variant led to less efficient processing of the miRNA precursor to its mature form as well as diminished capacity to regulate target genes. A whole-genome expression microarray was done and a pathway-based analysis identified a cancer-relevant network formed by genes significantly altered following enforced expression of miR-196a-2. Mutagenesis analysis further showed that cell cycle response to mutagen challenge was significantly enhanced in cells treated with variant miR-196a-2 compared with cells treated with the wild-type. Taken together, our findings suggest that miR-196a-2 might have a potentially oncogenic role in breast tumorigenesis, and the functional genetic variant in its mature region could serve as a novel biomarker for breast cancer susceptibility.

47. Negative Regulation of β_4 Integrin Transcription by Homeodomain-Interacting Protein Kinase 2 and p53 Impairs Tumor Progression, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5978-5986

ABSTRACT: Increased expression of $\alpha_6\beta_4$ integrin in several epithelial cancers promotes tumor progression; however, the mechanism underlying its transcriptional regulation remains unclear. Here, we show that depletion of homeodomain-interacting protein kinase 2 (HIPK2) activates β_4 transcription that results in a strong increase of β_4 -dependent mitogen-activated protein kinase and Akt phosphorylation, anchorage-independent growth, and invasion. In contrast, stabilization of HIPK2 represses β_4 expression in wild-type p53 (wtp53)-expressing cells but not in p53-null cells or cells expressing mutant p53, indicating that HIPK2 requires a wtp53 to inhibit β_4 transcription. Consistent with our in vitro findings, a strong correlation between β_4 overexpression and HIPK2 inactivation by cytoplasmic relocation was observed in wtp53-expressing human breast carcinomas. Under loss of function of HIPK2 or p53, the p53 family members TAp63 and TAp73 strongly activate β_4 transcription. These data, by revealing that β_4 expression is transcriptionally repressed in tumors by HIPK2 and p53 to impair β_4 -dependent tumor progression, suggest that loss of p53 function favors the formation of coactivator complex with the TA members of the p53 family to allow β_4 transcription.

48. Kinome Profiling in Pediatric Brain Tumors as a New Approach for Target Discovery, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5987-5995

ABSTRACT: Progression in pediatric brain tumor growth is thought to be the net result of signaling through various protein kinase-mediated networks driving cell proliferation. Defining new targets for treatment of human malignancies, without a priori knowledge on aberrant cell signaling activity, remains exceedingly complicated. Here, we introduce kinome profiling using flow-through peptide microarrays as a new concept for target discovery. Comprehensive tyrosine kinase activity profiles were identified in 29 pediatric brain tumors using the PamChip kinome profiling system. Previously reported activity of epidermal growth factor receptor, c-Met, and vascular endothelial growth factor receptor in pediatric brain tumors could be appreciated in our array results. Peptides corresponding with phosphorylation consensus sequences for Src family kinases showed remarkably high levels of phosphorylation compared with normal tissue types. Src activity was confirmed applying Phos-Tag SDS-PAGE. Furthermore, the Src family kinase inhibitors PP1 and dasatinib induced substantial tumor cell death in nine pediatric brain tumor cell lines but not in control cell lines. Thus, this study describes a new high-throughput technique to generate clinically relevant tyrosine kinase activity profiles as has been shown here for pediatric brain tumors. In the era of a rapidly increasing number of small-molecule inhibitors, this approach will enable us to rapidly identify new potential targets in a broad range of human malignancies.

49. Breast Cancer Lung Metastasis Requires Expression of Chemokine Receptor CCR4 and Regulatory T Cells, *Cancer Research*, July 15, 2009, Vol.69, Issue 14, pp 5996-6004

ABSTRACT: Cancer metastasis is a leading cause of cancer morbidity and mortality. More needs to be learned about mechanisms that control this process. In particular, the role of chemokine receptors in metastasis remains controversial. Here, using a highly metastatic breast cancer (4T1) model, we show that lung metastasis is a feature of only a proportion of the tumor cells that express CCR4. Moreover, the primary tumor growing in mammary pads activates remotely the expression of TARC/CCL17 and MDC/CCL22 in the lungs. These chemokines acting through CCR4 attract both tumor and immune cells. However, CCR4-mediated chemotaxis was not sufficient to produce metastasis, as tumor cells in the lung were efficiently eliminated by natural killer (NK) cells. Lung metastasis required CCR4⁺ regulatory T cells (Treg), which directly killed NK cells using β -galactoside-binding protein. Thus, strategies that abrogate any part of this process should improve the outcome through activation of effector cells and prevention of tumor cell migration. We confirm this prediction by killing CCR4⁺ cells through delivery of TARC-fused toxins or depleting Tregs and preventing lung metastasis.

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FROM JULY 20, 2009, VOLUME 27, ISSUE 21 OF JOURNAL OF CLINICAL ONCOLOGY

1. Expression of ER- α 36, a Novel Variant of Estrogen Receptor α , and Resistance to Tamoxifen Treatment in Breast Cancer, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3423-3429

PURPOSE AND METHODS: Recently, a 36-kDa variant of estrogen receptor α (ER- α 66), ER- α 36, has been identified and cloned. ER- α 36 predominantly localizes on the plasma membrane and in the cytoplasm and mediates a membrane-initiated "nongenomic" signaling pathway. Here, we investigate the association between ER- α 36 expression and tamoxifen resistance in patients with breast cancer. ER- α 36 protein expression in tumors from 896 women (two independent cohorts, 1 and 2) with operable primary breast cancer was assessed using an immunohistochemistry assay.

RESULTS AND CONCLUSION: In the first cohort of 710 consecutive patients, overexpression of ER- α 36 was associated with poorer disease-free survival (DFS) and disease-specific survival (DSS) in patients with ER- α 66–positive tumors who received tamoxifen treatment (chemotherapy plus tamoxifen or tamoxifen alone, $n = 307$). In contrast, ER- α 36 was not associated with survival in patients with ER- α 66–positive tumors who did not receive tamoxifen (chemotherapy alone, $n = 129$) and in patients with ER- α 66–negative tumors whether they received tamoxifen ($n = 73$) or not ($n = 149$). In the second cohort of 186 patients who only received tamoxifen as adjuvant therapy, overexpression of ER- α 36 was significantly associated with poorer DFS and DSS in 156 ER- α 66–positive patients from this cohort, and ER- α 36 remained an independent unfavorable factor for both DFS and DSS in these 156 patients by a multivariate analysis (DFS: hazard ratio [HR] = 5.47; 95% CI, 1.81 to 16.51; $P = .003$; DSS: HR = 13.97; 95% CI, 1.58 to 123.53; $P = .018$). Women with ER- α 66–positive tumors that also express high levels of ER- α 36 are less likely to benefit from tamoxifen treatment.

2. Topoisomerase II α Amplification Does Not Predict Benefit From Dose-Intense Cyclophosphamide, Doxorubicin, and Fluorouracil Therapy in *HER2*-Amplified Early Breast Cancer: Results of CALGB 8541/150013, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3430-3436

PURPOSE AND METHODS: We have demonstrated that patients with *HER2*-amplified tumors derive more benefit from higher doses of doxorubicin-containing chemotherapy (cyclophosphamide, doxorubicin, and fluorouracil [CAF]). Because topoisomerase II α (Topo-II α) is a target for doxorubicin and is coamplified in 20% to 50% of *HER2*-amplified tumors, we postulated that Topo-II α copy number might account for the benefit from CAF dose escalation in *HER2*-positive tumors. To address this hypothesis, we examined Topo-II α and *HER2* copy number, CAF dose, and clinical outcomes in Cancer and Leukemia Group B (CALGB) 8541. Topo-II α and *HER2* copy number were measured by fluorescent in situ hybridization (FISH) using a triple-probe system, which includes Topo-II α , *HER2*, and chromosome 17 (CEP17). Topo-II α and/or *HER2* were classified as amplified (\geq two copies/CEP17, deleted (\leq 0.67 copies/CEP17) and normal copy number ($> .67$ to < 2.0 copies/CEP17).

RESULTS AND CONCLUSION: Topo-II α /*HER2*/CEP17 measurement was successful in 624 of 687 cases. *HER2* was amplified in 117 cases (19%). Topo-II α was amplified in 41 cases (7%) and deleted in 69 cases (11%). Topo-II α amplification was highly correlated with *HER2* amplification (39 of 41; $P < .0001$), *HER2* by immunohistochemistry, and by dual-probe FISH. Topo-II α was deleted in both the *HER2*-amplified (30 of 69; 43%), normal (22 of 69; 32%) and *HER2*-deleted tumors (17 of 69; 25%). Although Topo-II α -amplified tumors were nearly always *HER2* amplified, these tumors did not receive benefit from increasing the dose of CAF ($P = .15$). The correlative companion study CALGB 8541-150013 does not support the hypothesis that Topo-II α amplification is the mechanism behind benefit from increased dose of anthracyclines in *HER2*-positive breast cancer.

3. Elevated Biomarkers of Inflammation Are Associated With Reduced Survival Among Breast Cancer Patients, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3437-3444

PURPOSE AND METHODS: Chronic inflammation is believed to contribute to the development and progression of breast cancer. Systemic C-reactive protein (CRP) and serum amyloid A (SAA) are measures of low-grade chronic inflammation and potential predictors of cancer survival. We evaluated the relationship between circulating markers of inflammation and breast cancer survival using data from the Health, Eating, Activity, and Lifestyle (HEAL) Study (a multiethnic prospective cohort study of women diagnosed with stage 0 to IIIA breast cancer). Circulating concentrations of CRP and SAA were measured approximately 31 months after diagnosis and tested for associations with disease-free survival (approximately 4.1 years of follow-up) and overall survival (approximately 6.9 years of follow-up) in 734 disease-free breast cancer survivors. Cox proportional hazards models were used with adjustment for potential confounding factors to generate hazard ratios (HRs) and 95% CIs.

RESULTS AND CONCLUSION: Elevated SAA and CRP were associated with reduced overall survival, regardless of adjustment for age, tumor stage, race, and body mass index (SAA P trend < .0001; CRP P trend = .002). The HRs for SAA and CRP tertiles suggested a threshold effect on survival, rather than a dose-response relationship (highest v lowest tertile: SAA HR = 3.15; 95% CI, 1.73 to 5.65; CRP HR = 2.27; 95% CI, 1.27 to 4.08). Associations were similar and still significant after adjusting for self-reported history of cardiovascular events and censoring cardiovascular disease deaths. Elevated CRP and SAA were also associated with reduced disease-free survival, although these associations were of borderline significance (SAA P trend = .04; CRP P trend = .07). Circulating SAA and CRP may be important prognostic markers for long-term survival in breast cancer patients, independent of race, tumor stage, and body mass index.

4. Adjuvant Hormonal Therapy Use Among Insured, Low-Income Women With Breast Cancer, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3445-3451

PURPOSE AND METHODS: Use of adjuvant hormonal therapy, which significantly decreases breast cancer mortality, has not been well described among poor women, who are at higher risk of cancer-related death. Here we explore use of adjuvant hormonal therapy in an insured, low-income population. A North Carolina Cancer Registry–Medicaid linked data set was used. Women with hormone receptor–positive or unknown, nonmetastatic breast cancer, diagnosed between 1998 and 2002, were included. Main outcomes were (1) prescription fill within 1 year of diagnosis, (2) adherence (medication possession ratio), and (3) persistence (absence of a 90-day gap in prescription fills over 12 months).

RESULTS AND CONCLUSION: The population consisted of 1,491 women (mean age, 67 years). Sixty-four percent filled prescriptions. Predictors of prescription fill included the following: older age (odds ratio [OR], 1.01; P = .017), greater number of prescription medications (OR, 1.06; P < .001), nonmarried status (OR, 1.82; P = .001), higher stage (OR, 1.83; P < .001), positive hormone receptor status (positive v unknown, OR, 1.98; P < .001), not receiving adjuvant chemotherapy (OR, 1.74; P = .001), receipt of adjuvant radiation (OR, 1.55; P = .004), and treatment in a small hospital (OR, 1.49; P = .024). Adherence and persistence rates were 60% and 80%, respectively. Nonmarried status predicted greater adherence (OR, 1.90; P = .006) and persistence (OR, 1.75; P = .031). Prescription fill, adherence, and persistence to adjuvant hormonal therapy among socioeconomically disadvantaged women are low. Improving use of adjuvant hormonal therapy may lead to lower breast cancer–specific mortality in this population.

5. Impact of Androgen Deprivation Therapy on Cardiovascular Disease and Diabetes, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3452-3458

PURPOSE AND METHODS: Use of androgen deprivation therapy (ADT) may be associated with an increased risk of diabetes mellitus but the risk of both acute myocardial infarction (AMI) and cardiovascular mortality remain controversial because few outcomes and conflicting findings have been reported. We sought to clarify whether ADT is associated with these outcomes in a large, representative cohort. Using linked administrative databases in Ontario, Canada, men age 66 years or older with prostate cancer given continuous ADT for at least 6 months or who underwent bilateral orchiectomy (n = 19,079) were matched with men with prostate cancer who had never received ADT. Treated and untreated groups were matched 1:1 (ie, hard-matched) on age, prior cancer treatment, and year of diagnosis and propensity-matched on comorbidities, medications, cardiovascular risk factors, prior fractures, and socioeconomic variables. Primary outcomes were development of AMI, sudden cardiac death, and diabetes. Fragility fracture was also examined.

RESULTS AND CONCLUSION: The cohort was observed for a mean of 6.47 years. In time-to-event analyses, ADT use was associated with an increased risk of diabetes (hazard ratio [HR], 1.16; 95% CI, 1.11 to 1.21) and fragility fracture (HR, 1.65; 95% CI, 1.53 to 1.77) but not with AMI (HR, 0.91; 95% CI, 0.84 to 1.00) or sudden cardiac death (HR, 0.96; 95% CI, 0.83 to 1.10). Increasing duration of ADT was associated with an excess risk of fragility fractures and diabetes but not cardiac outcomes. Continuous ADT use for at least 6 months in older men is associated with an increased risk of diabetes and fragility fracture but not AMI or sudden cardiac death.

6. Gleason Score and Lethal Prostate Cancer: Does $3 + 4 = 4 + 3$? , Journal of Clinical Oncology, July 20, 2009, Vol. 27, Issue 21, pp 3459-3464

PURPOSE AND METHODS: Gleason grading is an important predictor of prostate cancer (PCa) outcomes. Studies using surrogate PCa end points suggest outcomes for Gleason score (GS) 7 cancers vary according to the predominance of pattern 4. These studies have influenced clinical practice, but it is unclear if rates of PCa mortality differ for $3 + 4$ and $4 + 3$ tumors. Using PCa mortality as the primary end point, we compared outcomes in Gleason $3 + 4$ and $4 + 3$ cancers, and the predictive ability of GS from a standardized review versus original scoring. Three study pathologists conducted a blinded standardized review of 693 prostatectomy and 119 biopsy specimens to assign primary and secondary Gleason patterns. Tumor specimens were from PCa patients diagnosed between 1984 and 2004 from the Physicians' Health Study and Health Professionals Follow-Up Study. Lethal PCa ($n = 53$) was defined as development of bony metastases or PCa death. Hazard ratios (HR) were estimated according to original GS and standardized GS. We compared the discrimination of standardized and original grading with C-statistics from models of 10-year survival.

RESULTS AND CONCLUSION: For prostatectomy specimens, $4 + 3$ cancers were associated with a three-fold increase in lethal PCa compared with $3 + 4$ cancers (95% CI, 1.1 to 8.6). The discrimination of models of standardized scores from prostatectomy (C-statistic, 0.86) and biopsy (C-statistic, 0.85) were improved compared to models of original scores (prostatectomy C-statistic, 0.82; biopsy C-statistic, 0.72). Ignoring the predominance of Gleason pattern 4 in GS 7 cancers may conceal important prognostic information. A standardized review of GS can improve prediction of PCa survival.

7. Conversion to Resectability Using Hepatic Artery Infusion Plus Systemic Chemotherapy for the Treatment of Unresectable Liver Metastases From Colorectal Carcinoma, Journal of Clinical Oncology, July 20, 2009, Vol. 27, Issue 21, pp 3465-3471

PURPOSE AND METHODS: To determine the conversion to resectability in patients with unresectable liver metastases from colorectal cancer treated with hepatic arterial infusion (HAI) plus systemic oxaliplatin and irinotecan (CPT-11). Forty-nine patients with unresectable liver metastases (53% previously treated with chemotherapy) were enrolled onto a phase I protocol with HAI floxuridine and dexamethasone plus systemic chemotherapy with oxaliplatin and irinotecan.

RESULTS AND CONCLUSION: Ninety-two percent of the 49 patients had complete (8%) or partial (84%) response, and 23 (47%) of the 49 patients were able to undergo resection in a group of patients with extensive disease (73% with > five liver lesions, 98% with bilobar disease, 86% with \geq six segments involved). For chemotherapy-naïve and previously treated patients, the median survival from the start of HAI therapy was 50.8 and 35 months, respectively. The only baseline variable significantly associated with a higher resection rate was female sex. Variables reflecting extensive anatomic disease, such as number of lesions or number of vessels involved, were not significantly associated with the probability of resection. The combination of regional HAI floxuridine/dexamethasone and systemic oxaliplatin and irinotecan is an effective regimen for the treatment of patients with unresectable liver metastases from colorectal cancer, demonstrating a 47% conversion to resection (57% in chemotherapy-naïve patients). Future randomized trials should compare HAI plus systemic chemotherapy with systemic therapy alone to assess the additional value of HAI therapy in converting patients with hepatic metastases to respectability.

8. Dasatinib in the Treatment of Chronic Myeloid Leukemia in Accelerated Phase After Imatinib Failure: The START A Trial, Journal of Clinical Oncology, July 20, 2009, Vol. 27, Issue 21, pp 3472-3479

PURPOSE AND METHODS: Patients with chronic myelogenous leukemia in accelerated phase (CML-AP) that is resistant or intolerant to imatinib have limited therapeutic options. Dasatinib, a potent inhibitor of BCR-ABL and SRC-family kinases, has efficacy in patients with CML-AP who have experienced treatment failure with imatinib. We now report follow-up data from the full patient cohort of 174 patients enrolled onto a phase II trial to provide a more complete assessment of the efficacy and safety of dasatinib in this population. Patients with imatinib-resistant ($n = 161$) or -intolerant ($n = 13$) CML-AP received dasatinib 70 mg orally twice daily.

RESULTS AND CONCLUSION: At a median follow-up of 14.1 months (treatment duration, 0.1 to 21.7 months), major and complete hematologic responses were attained by 64% and 45% of patients, respectively, and major and complete cytogenetic responses were achieved in 39% and 32% of patients, respectively. Responses were achieved irrespective of imatinib status (resistant or intolerant), prior stem-cell transplantation, or the presence of prior BCR-ABL mutation. The 12-month progression-free survival and overall survival rates were 66% and 82%, respectively. Dasatinib was generally well tolerated; the most frequent nonhematologic severe treatment-related adverse event was diarrhea (52%; grade 3 to 4, 8%). Cytopenias were common, including grade 3 to 4 neutropenia (76%) and thrombocytopenia (82%). Pleural effusion occurred in 27% of patients (grade 3 to 4, 5%). Dasatinib is effective in patients with CML-AP after imatinib treatment failure.

9. Dose-Modified Oral Chemotherapy in the Treatment of AIDS-Related Non-Hodgkin's Lymphoma in East Africa, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3480-3488

PURPOSE AND METHODS: Africa is burdened by the AIDS epidemic and attendant increase in HIV/AIDS-related malignancies. Pragmatic approaches to therapeutic intervention could be of great value. Dose-modified oral chemotherapy for AIDS-related non-Hodgkin's lymphoma is one such approach. The oral regimen consisted of lomustine 50 mg/m² on day 1 (cycle 1 only), etoposide 100 mg/m² on days 1 to 3, and cyclophosphamide/procarbazine 50 mg/m² each on days 22 to 26 at 6-week intervals (one cycle) for two total cycles in HIV-infected patients with biopsy-proven non-Hodgkin's lymphoma.

RESULTS AND CONCLUSION: Forty-nine patients (21 in Uganda and 28 in Kenya) were treated. The majority of patients were female (59%) and had a poor performance status (63%); 69% of patients had advanced-stage disease; and 18 patients (37%) had access to antiretroviral therapy. In total, 79.5 cycles of therapy were administered. The regimen was well tolerated, had modest effects (decline) on CD4⁺ lymphocyte counts ($P = .077$), and had negligible effects on HIV-1 viral replication. Four febrile neutropenia episodes and three treatment-related deaths (6% mortality rate) occurred. The overall objective response rate was 78% (95% CI, 62% to 88%); median follow-up time was 8.2 months (range, 0.1 to 71 months); median event-free and overall survival times were 7.9 months (95% CI, 3.3 to 13.0 months) and 12.3 months (95% CI, 4.9 to 32.4 months), respectively; and 33% of patients survived 5 years. Dose-modified oral chemotherapy is efficacious, has comparable outcome to that in the United States in the pre–highly active antiretroviral therapy setting, has an acceptable safety profile, and is pragmatic in sub-Saharan Africa. The international collaboration has been highly successful, and subsequent projects should focus on strategies to optimize combination antiretroviral therapy and chemotherapy and follow-up tissue correlative studies.

10. Improved Survival for Stage IV Melanoma From an Unknown Primary Site, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3489-3495

PURPOSE AND METHODS: We previously demonstrated a survival advantage for nodal metastasis of melanoma from an unknown primary (MUP) versus melanoma from a known primary (MKP). We hypothesized that this survival benefit would extend to MUP patients with distant (stage IV) metastasis. We reviewed prospectively acquired data for 2,247 patients diagnosed with American Joint Committee on Cancer stage IV melanoma at our cancer center between 1971 and 2005. Cox regression analysis in a multivariate model identified prognostic factors significant for survival. MUP and MKP patients were then matched by significant covariates. Overall survival (OS) was estimated by Kaplan-Meier method and compared by log-rank analysis.

RESULTS AND CONCLUSION: There were 1,849 MKP and 398 MUP patients. Multivariate analysis of patients with complete data sets identified known/unknown primary (hazard ratio [HR], 1.141; $P = .032$) and five other significant covariates: age (HR, 1.148; $P = .007$), sex (HR, 1.17; $P = .001$), site of metastasis (HR, 1.336; $P < .001$), number of different metastatic sites (HR, 1.303; $P < .001$), and decade of diagnosis (HR, 0.713; $P < .001$). Prognostic matching yielded 392 MUP-MKP pairs. Median OS and 5-year OS rate were significantly greater ($P < .001$) for MUP patients than for all matched MKP patients or for MKP patients matched by M1 category (for M1b and M1c) or number of metastatic sites. The survival advantage previously reported for patients with stage III MUP also applies to patients with stage IV MUP. The mechanism responsible for this improved survival may provide clues for more effective treatment of stage IV melanoma and therefore warrants further investigation. The improved results for MUP suggest that these patients deserve aggressive therapy.

11. Prospective Randomized Multicenter Adjuvant Dermatologic Cooperative Oncology Group Trial of Low-Dose Interferon Alfa-2b With or Without a Modified High-Dose Interferon Alfa-2b Induction Phase in Patients With Lymph Node–Negative Melanoma, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3496-3502

PURPOSE AND METHODS: Interferon alfa (IFN- α) has shown clinical efficacy in the adjuvant treatment of patients with high-risk melanoma in several clinical trials, but optimal dosing and duration of treatment are still under discussion. It has been argued that in high-dose IFN- α (HDI), the intravenous (IV) induction phase might be critical for the clinical benefit of the regimen. In an attempt to investigate the potential role of a modified high-dose induction phase, lymph node–negative patients with resected primary malignant melanoma of more than 1.5-mm tumor thickness were included in this prospective randomized multicenter Dermatologic Cooperative Oncology Group trial. Six hundred seventy-four patients were randomly assigned to receive 4 weeks of a modified HDI scheme. This schedule consisted of 5 times weekly 10 MU/m² IFN- α -2b IV for 2 weeks and 5 times weekly 10 MU/m² IFN- α -2b administered subcutaneously (SC) for another 2 weeks followed by 23 months of low-dose IFN- α -2b (LDI) 3 MU SC three times a week (arm A). LDI 3 MU three times a week was given for 24 months in arm B.

RESULTS AND CONCLUSION: Of 650 assessable patients, there were 92 relapses among the 321 patients receiving high-dose induction as compared with 95 relapses among the 329 patients receiving LDI only. Five-year relapse-free survival rates were 68.0% (arm A) and 67.1% (arm B), respectively. Likewise, melanoma-related fatalities were similar between both groups, resulting in 5-year overall survival rates of 80.2% (arm A) and 82.9% (arm B). The addition of a 4-week modified HDI induction phase to a 2-year low-dose adjuvant IFN- α -2b treatment schedule did not improve the clinical outcome.

12. Blood-Brain Barrier Disruption and Intra-Arterial Methotrexate-Based Therapy for Newly Diagnosed Primary CNS Lymphoma: A Multi-Institutional Experience, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3503-3509

PURPOSE AND METHODS: Primary CNS lymphoma (PCNSL) is confined to the CNS and/or the eyes at presentation and is usually initially treated with intravenous methotrexate-based chemotherapy and whole-brain radiotherapy (WBRT). However, the intact blood-brain barrier (BBB) can limit diffusion of methotrexate into brain and tumor. With BBB disruption (BBBD), enhanced drug delivery to the tumor can be achieved. This report summarizes the multi-institutional experience of 149 newly diagnosed (with no prior WBRT) patients with PCNSL treated with osmotic BBBD and intra-arterial (IA) methotrexate at four institutions from 1982 to 2005. In this series, 47.6% of patients were age \geq 60 years, and 42.3% had Karnofsky performance score (KPS) less than 70 at diagnosis.

RESULTS AND CONCLUSION: The overall response rate was 81.9% (57.8% complete; 24.2% partial). Median overall survival (OS) was 3.1 years (25% estimated survival at 8.5 years). Median progression-free survival (PFS) was 1.8 years, with 5-year PFS of 31% and 7-year PFS of 25%. In low-risk patients (age < 60 years and KPS \geq 70), median OS was approximately 14 years, with a plateau after approximately 8 years. Procedures were generally well tolerated; focal seizures (9.2%) were the most frequent side effect and lacked long-term sequelae. This large series of patients treated over a 23-year period demonstrates that BBBD/IA methotrexate-based chemotherapy results in successful and durable tumor control and outcomes that are comparable or superior to other PCNSL treatment regimens.

13. Phase II Study of Thalidomide Plus Dexamethasone Induction Followed by Tandem Melphalan-Based Autotransplantation and Thalidomide-Plus-Prednisone Maintenance for Untreated Multiple Myeloma: A Southwest Oncology Group Trial (S0204), *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3510-3517

PURPOSE AND METHODS: Thalidomide-dexamethasone (THAL-DEX) is standard induction therapy for multiple myeloma (MM). Tandem melphalan-based transplantations have yielded superior results to single transplantations. Phase II trial S0204 was designed to improve survival results reported for the predecessor, phase III trial S9321 by 50%. Newly diagnosed patients with MM were eligible for S0204 with THAL-DEX induction, tandem melphalan-based tandem transplantation, and THAL-prednisone maintenance.

RESULTS AND CONCLUSION: Of 143 eligible patients, 142 started induction, 73% completed first transplantation, 58% completed second transplantation, and 56% started maintenance. The quantity of stem cells required for two transplantations was reached in 88% of 111 patients undergoing collection, 74% of whom completed both transplantations. Partial response, very good partial remission, and complete response were documented after 12 months of maintenance therapy in 87%, 72%, and 22% of patients, respectively. During a median follow-up time of 37 months, 4-year estimates of event-free and overall survival were 50% and 64%, respectively. Survival outcomes were superior for International Staging System (ISS) stage 1 disease, when lactate dehydrogenase (LDH) levels were normal and a second transplantation was applied in a timely fashion. Both overall survival ($P = .0002$) and event-free survival ($P < .0001$) were significantly improved with S0204 compared with S9321 when 121 and 363 patients, respectively, were matched on ISS stage and LDH.

14. Single-Agent Bortezomib in Previously Untreated Multiple Myeloma: Efficacy, Characterization of Peripheral Neuropathy, and Molecular Correlations With Response and Neuropathy, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3518-3525

PURPOSE AND METHODS: To assess efficacy and safety of single-agent bortezomib in previously untreated patients with multiple myeloma, investigate prevalence of baseline and treatment-emergent polyneuropathy, and identify molecular markers associated with response and neuropathy. Patients received bortezomib 1.3 mg/m² on days 1, 4, 8, and 11, for up to eight 21-day cycles. A subset of patients underwent neurophysiologic evaluation pre- and post-treatment. Bone marrow aspirates were performed at baseline for exploratory whole-genome analyses.

RESULTS AND CONCLUSION: Among 64 patients, 41% had partial response or better, including 9% complete/near-complete responses; median duration of response was 8.4 months. Response rates did not differ in the presence or absence of adverse cytogenetics. After median follow-up of 29 months, median time to progression was 17.3 months. Median overall survival had not been reached; estimated 1-year survival was 92%. Thirty-two patients successfully underwent optional stem-cell transplantation. Bortezomib treatment was generally well tolerated. At baseline, 20% of patients had sensory polyneuropathy. Sensory polyneuropathy developed during treatment in 64% of patients (grade 3 in 3%), but proved manageable and resolved in 85% within a median of 98 days. Neurologic examination, neurophysiologic testing, and measurements of epidermal nerve fiber densities in 35 patients confirmed pretreatment sensory neuropathy in 20% and new or worsening neuropathy in 63%. Pharmacogenomic analyses identified molecular markers of response and treatment-emergent neuropathy, which will require future study. Single-agent bortezomib is effective in previously untreated myeloma. Baseline myeloma-associated neuropathy seems more common than previously reported, and bortezomib-associated neuropathy, although a common toxicity, is reversible in most patients.

15. Cognitive Deficits and Predictors 3 Years After Diagnosis of a Pilocytic Astrocytoma in Childhood, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3526-3532

PURPOSE AND METHODS: To prospectively study cognitive deficits and predictors 3 years after diagnosis in a large series of pediatric patients treated for pilocytic astrocytoma (PA). Sixty-one of 67 children were grouped according to infratentorial, supratentorial midline, and supratentorial hemispheric site. Intelligence, memory, attention, language, visual-spatial, and executive functions were assessed. Included predictors were sex, age, relapse, diagnosis-assessment interval, hydrocephalus, kind of treatment, and tumor variables.

RESULTS AND CONCLUSION: All children with PA had problems with sustained attention and speed. In the infratentorial group, there also were deficits in verbal intelligence, visual-spatial memory, executive functioning, and naming. Verbal intelligence and verbal memory problems occurred in the brainstem tumor group. The supratentorial hemispheric tumor group had additional problems with selective attention and executive functioning, and the supratentorial midline tumor group displayed no extra impairments. More specifically, the dorsal supratentorial midline tumor group displayed problems with language and verbal memory. Predictors for lower cognitive functioning were hydrocephalus, radiotherapy, residual tumor size, and age; predictors for better functioning were chemotherapy or treatment of hydrocephalus. Almost 60% of children had problems with academic achievement, for which risk factors were relapse and younger age at diagnosis. Despite normal intelligence at long-term follow-up, children treated for PA display invalidating cognitive impairments. Adequate treatment of hydrocephalus is important for a more favorable long-term cognitive outcome. Even children without initial severe deficits may develop cognitive impairments years after diagnosis, partly because of the phenomenon of growing into deficit, which has devastating implications for academic achievement and quality of life (QOL).

16. Minimal Disseminated Disease in Childhood T-Cell Lymphoblastic Lymphoma: A Report From the Children's Oncology Group, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3533-3539

PURPOSE AND METHODS: Disease dissemination to the bone marrow is detected at diagnosis in approximately 15% of children with T-cell lymphoblastic lymphoma (T-LL). It is unclear whether the remaining patients have submicroscopic systemic disease and, if so, what is the clinical significance of this finding. Using a flow cytometric method that can detect one T-LL cell among 10,000 normal cells, we examined bone marrow and peripheral-blood samples collected from 99 children with T-LL at diagnosis, as well as blood samples collected from 42 patients during treatment.

RESULTS AND CONCLUSION: In 71 (71.7%) of the 99 marrow samples obtained at diagnosis, T-LL cells represented 0.01% to 31.6% (median, 0.22%) of mononuclear cells; 57 of the 71 T-LL-positive samples were from patients with stage II/III disease. Results of studies in bilateral marrow aspirates were highly concordant. Two-year event-free survival (EFS) was $68.1\% \pm 11.1\%$ (SE) for patients with $\geq 1\%$ T-LL cells in bone marrow versus $90.7\% \pm 4.4\%$ for those with lower levels of marrow involvement ($P = .031$); EFS for patients with $\geq 5\%$ lymphoblasts was $51.9\% \pm 18.0\%$ ($P = .009$). T-LL cells were as prevalent in blood as in marrow; monitoring residual T-LL cells in blood during remission induction therapy identified patients with slower disease clearance. More than two thirds of children with T-LL have disseminated disease at diagnosis, a proportion much higher than previously demonstrated. Measurements of disease dissemination at diagnosis might provide useful prognostic information, which can be further refined by monitoring response to therapy through blood testing.

17. Japanese-US Common-Arm Analysis of Paclitaxel Plus Carboplatin in Advanced Non-Small-Cell Lung Cancer: A Model for Assessing Population-Related Pharmacogenomics, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3540-3546

PURPOSE AND METHODS: To explore whether population-related pharmacogenomics contribute to differences in patient outcomes between clinical trials performed in Japan and the United States, given similar study designs, eligibility criteria, staging, and treatment regimens. We prospectively designed and conducted three phase III trials (Four-Arm Cooperative Study, LC00-03, and S0003) in advanced-stage, non-small-cell lung cancer, each with a common arm of paclitaxel plus carboplatin. Genomic DNA was collected from patients in LC00-03 and S0003 who received paclitaxel (225 mg/m^2) and carboplatin (area under the concentration-time curve, 6). Genotypic variants of CYP3A4, CYP3A5, CYP2C8, NR1I2-206, ABCB1, ERCC1, and ERCC2 were analyzed by pyrosequencing or by PCR restriction fragment length polymorphism. Results were assessed by Cox model for survival and by logistic regression for response and toxicity.

RESULTS AND CONCLUSION: Clinical results were similar in the two Japanese trials, and were significantly different from the US trial, for survival, neutropenia, febrile neutropenia, and anemia. There was a significant difference between Japanese and US patients in genotypic distribution for CYP3A4*1B ($P = .01$), CYP3A5*3C ($P = .03$), ERCC1 118 ($P < .0001$), ERCC2 K751Q ($P < .001$), and CYP2C8 R139K ($P = .01$). Genotypic associations were observed between CYP3A4*1B for progression-free survival (hazard ratio [HR], 0.36; 95% CI, 0.14 to 0.94; $P = .04$) and ERCC2 K751Q for response (HR, 0.33; 95% CI, 0.13 to 0.83; $P = .02$). For grade 4 neutropenia, the HR for ABCB1 3425C→T was 1.84 (95% CI, 0.77 to 4.48; $P = .19$). Differences in allelic distribution for genes involved in paclitaxel disposition or DNA repair were observed between Japanese and US patients. In an exploratory analysis, genotype-related associations with patient outcomes were observed for CYP3A4*1B and ERCC2 K751Q. This common-arm approach facilitates the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.

18. Quality of Life After Pelvic Radiotherapy or Vaginal Brachytherapy for Endometrial Cancer: First Results of the Randomized PORTEC-2 Trial, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3547-3556

PURPOSE AND METHODS: Studies on quality of life (QOL) among women with endometrial cancer have shown that patients who undergo pelvic radiotherapy report lower role functioning and more diarrhea and fatigue. In the Post Operative Radiation Therapy in Endometrial Cancer (PORTEC) trial, patients with endometrial carcinoma were randomly assigned to receive external-beam radiotherapy (EBRT) or vaginal brachytherapy (VBT). QOL was evaluated by using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30 and subscales from the prostate cancer module, PR-25, and the ovarian cancer module, OV-28. PORTEC-2 accrued 427 patients between 2002 and 2006, of whom 214 were randomly assigned to EBRT, and 213 were randomly assigned to VBT. Three-hundred forty-eight patients (81%) were evaluable for QOL. QOL outcomes were analyzed at a median follow-up of 2 years.

RESULTS AND CONCLUSION: At baseline after surgery, patient functioning was at the lowest level, and it increased during and after radiotherapy to reach a plateau after 12 months. Patients in the VBT group reported better social functioning ($P < .002$) and lower symptom scores for diarrhea, fecal leakage, the need to stay close to the toilet, and limitation in daily activities because of bowel symptoms ($P < .001$). At baseline, 15% of patients were sexually active; this increased significantly to 39% during the first year ($P < .001$). Sexual functioning and symptoms did not differ between the treatment groups. Patients who received EBRT reported significantly higher levels of diarrhea and bowel symptoms. This resulted in a higher need to remain close to a toilet and, as a consequence, more limitation of daily activities because of bowel symptoms and decreased social functioning. Vaginal brachytherapy provides a better QOL, and should be the preferred treatment from a QOL perspective.

19. Safety, Pharmacokinetics, and Antitumor Activity of AMG 386, a Selective Angiopoietin Inhibitor, in Adult Patients With Advanced Solid Tumors, *Journal of Clinical Oncology*, July 20, 2009, Vol. 27, Issue 21, pp 3557-3565

PURPOSE AND METHODS: AMG 386 is an investigational peptide-Fc fusion protein (ie, peptibody) that inhibits angiogenesis by preventing the interaction of angiopoietin-1 and angiopoietin-2 with their receptor, Tie2. This first-in-human study evaluated the safety, pharmacokinetics (PK), pharmacodynamics, and antitumor activity of AMG 386 in adults with advanced solid tumors. Patients in sequential cohorts received weekly intravenous AMG 386 doses of 0.3, 1, 3, 10, or 30 mg/kg.

RESULTS AND CONCLUSION: Thirty-two patients were enrolled on the study and received AMG 386. One occurrence of dose-limiting toxicity was seen at 30 mg/kg: respiratory arrest, which likely was caused by tumor burden that was possibly related to AMG 386. The most common toxicities were fatigue and peripheral edema. Proteinuria ($n = 11$) was observed without clinical sequelae. Only four patients (12%) experienced treatment-related toxicities greater than grade 1. A maximum-tolerated dose was not reached. PK was dose-linear and the mean terminal-phase elimination half-life values ranged from 3.1 to 6.3 days. Serum AMG 386 levels appeared to reach steady-state after four weekly doses, and there was minimal accumulation. No anti-AMG 386 neutralizing antibodies were detected. Reductions in volume transfer constant (K^{trans} ; measured by dynamic contrast-enhanced magnetic resonance imaging) were observed in 10 patients (13 lesions) 48 hours to 8 weeks after treatment. One patient with refractory ovarian cancer achieved a confirmed partial response (ie, 32.5% reduction by Response Evaluation Criteria in Solid Tumors) and withdrew from the study with a partial response after 156 weeks of treatment; four patients experienced stable disease for at least 16 weeks. Weekly AMG 386 appeared well tolerated, and its safety profile appeared distinct from that of vascular endothelial growth factor-axis inhibitors. AMG 386 also appeared to impact tumor vascularity and showed antitumor activity in this patient population.

FROM JULY 15, 2009, VOLUME 15, ISSUE 14 OF *CLINICAL CANCER RESEARCH*

1. Revealing Tumor Immunity after Hematopoietic Stem Cell Transplantation, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4515-4517

ABSTRACT: Allogeneic hematopoietic stem cell transplantation is currently the most effective method for inducing tumor immunity. However, the diversity of target antigens recognized by donor T cells has not been established. New studies show that tumor-reactive T cells are directed against diverse tumor-specific targets as well as minor histocompatibility antigens.

2. Inhibition of PI3K and MEK: It Is All about Combinations and Biomarkers, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4518-4520

ABSTRACT: A small molecule inhibitor of MAP/ERK kinase (MEK) was effective against human breast cancer cells with a basal-like gene expression signature. Antitumor activity was limited by both feedback upregulation of phosphatidylinositol-3 kinase (PI3K)/AKT upon inhibition of MEK as well as loss of the phosphatase PTEN. Therefore, MEK inhibitors should preferably be investigated in combination with PI3K inhibitors in basal-like breast cancers. (*Clin Cancer Res* 2009;15(14) July 2009).

3. Endothelin B Receptor, a New Target in Cancer Immune Therapy, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4521-4528

ABSTRACT: The endothelins and their G protein-coupled receptors A and B have been implicated in numerous diseases and have recently emerged as pivotal players in a variety of malignancies. Tumors overexpress the endothelin 1 (ET-1) ligand and the endothelin-A-receptor (ET_AR). Their interaction induces tumor growth and metastasis by promoting tumor cell survival and proliferation, angiogenesis, and tissue remodeling. On the basis of results from xenograft models, drug development efforts have focused on antagonizing the autocrine-paracrine effects mediated by ET-1/ET_AR. In this review, we discuss a novel role of the endothelin-B-receptor (ET_BR) in tumorigenesis and the effect of its blockade during cancer immune therapy. We highlight key characteristics of the B receptor such as its specific overexpression in the tumor compartment; and specifically, in the tumor endothelium, where its activation by ET-1 suppresses T-cell adhesion and homing to tumors. We also review our recent findings on the effects of ET_BR-specific blockade in increasing T-cell homing to tumors and enhancing the efficacy of otherwise ineffective immunotherapy.

4. Down-regulation of *Epidermal Growth Factor Receptor* by Selective Expansion of a 5'-End Regulatory Dinucleotide Repeat in Colon Cancer with Microsatellite Instability, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4531-4537

PURPOSE AND METHODS: The epidermal growth factor receptor (EGFR) is overexpressed in several tumor types, and its expression is influenced by the length of a 5'-end microsatellite repeat (CA)_n: the longer the repeat, the lower the expression. Dinucleotide repeats accumulate insertion/deletion types of mutations in tumors with microsatellite instability. We designed this study to estimate the occurrence of these mutations in EGFR(CA)_n and their relevance in carcinogenesis of microsatellite instability-positive colon and gastric tumors. We analyzed the frequency of EGFR(CA)_n mutations in vivo in 55 colorectal and 14 gastric microsatellite instability-positive cancers, and in vitro in single-cell clone cultures of microsatellite instability-positive colon tumor cell line LS174. Single-cell clone cultures with different repeat lengths were analyzed by fluorescent-activated cell sorter for EGFR cell-surface expression. A correlation analysis was done between EGFR(CA)_n mutations and mutations in KRAS, BRAF, and p53.

RESULTS AND CONCLUSION: Unlike single-cell clone cultures, which exhibited higher rate of deletions compared with insertions, most of EGFR(CA)_n mutations in colon and gastric tumors were insertions. Longer EGFR(CA)_n correlated with lower EGFR cell-surface expression in single-cell clone cultures. In colon cancers, the elongation of the repeat was associated negatively with mutations in KRAS and BRAF, but not in p53. The EGFR(CA)_n elongation observed in tumors cannot be explained by an intrinsic property of this repeat favoring insertions versus deletions. Instead, a selection for repeat elongation occurs in microsatellite instability-positive tumors, leading to EGFR down-regulation. These findings suggest that in microsatellite instability-positive tumors current therapies targeting EGFR overexpression may have either no effect or an opposite to the expected effect.

5. Activation of Phosphatidylinositol-3'-kinase/AKT Signaling Is Essential in Hepatoblastoma Survival, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4538-4545

PURPOSE AND METHODS: Hepatoblastoma represents the most frequent malignant liver tumor in childhood. The phosphatidylinositol-3'-kinase (PI3K)/AKT pathway is crucial in downstream signaling of multiple receptor tyrosine kinases of pathogenic importance in hepatoblastoma. Increased PI3K/AKT signaling pathway activity and activating mutations of PIK3CA, encoding a PI3K catalytic subunit, have been reported in different childhood tumors. The current study was done to analyze the role of PI3K/AKT signaling in hepatoblastoma. Immunohistochemical stainings of (Ser473)-phosphorylated (p)-AKT protein, its targets p-(Ser9)-GSK-3 β and p-(Ser2448)-mTOR, as well as the cell cycle regulators Cyclin D1, p27^{KIP1}, and p21^{CIP1} were done and the PIK3CA gene was screened for mutations. In vitro, two hepatoblastoma cell lines treated with the PI3K inhibitor LY294002 were analyzed for AKT and GSK-3 β phosphorylation, cell proliferation, and apoptosis. Additionally, simultaneous treatments of hepatoblastoma with LY294002 and cytotoxic drugs were carried out.

RESULTS AND CONCLUSION: Most tumors strongly expressed p-AKT, p-GSK-3 β , and p-mTOR; subgroups showed significant Cyclin D1, p27^{KIP1}, and p21^{CIP1} expression. One hepatoblastoma carried an E545A mutation in the PIK3CA gene. In vitro, PI3K inhibition diminished hepatoblastoma cell growth being accompanied by reduced AKT and GSK-3 β phosphorylation. Flow cytometry and 4', 6-diamidino-2-phenylindole stainings showed that PI3K pathway inhibition leads to a substantial increase in apoptosis and a decrease in cellular proliferation linked to reduced Cyclin D1 and increased p27^{KIP1} levels. Simultaneous treatment of hepatoblastoma cell lines with LY294002 and cytotoxic drugs resulted in positive interactions. Our findings imply that PI3K signaling plays an essential role in growth control of hepatoblastoma and might be successfully targeted in multimodal therapeutic strategies.

6. Regulation of Replicative and Stress-Induced Senescence by RSK4, which is Down-regulated in Human Tumors, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4546-4553

PURPOSE AND METHODS: The control of senescence and its biochemical pathways is a crucial factor for understanding cell transformation. In a large RNA interference screen, the RSK4 gene was found to be related to p53-dependent arrest. The purpose of the present study was to investigate the potential role of RSK4 as a tumor suppressor gene. RSK4 expression was determined by quantitative real-time PCR and immunoblot in 30 colon and 20 renal carcinomas, and in 7 colon adenomas. Two HCT116 colon carcinoma cell lines (p53 wt and p53 null), IMR90 human fibroblasts, and E1A-expressing IMR90 cells were infected with RSK4 cDNA and/or shRNA. RSK4 expression levels were analyzed in HCT116 p53 wt or p53 null and IMR90 after senescence induction by quantitative real-time PCR and Western blot.

RESULTS AND CONCLUSION: The RSK4 gene was down-regulated in 27 of 30 colon carcinomas ($P < 0.001$), 16 of 20 renal cell carcinomas ($P < 0.01$), and 6 of 7 colon adenomas ($P < 0.01$). In vitro overexpression of RSK4 induced cell arrest and senescence features in normal fibroblasts and malignant colon carcinoma cell lines. Interestingly, in these cell lines RSK4 mRNA levels were increased both in replicative and stress-induced senescence. Moreover, IMR90 partially immortalized by RSK4 shRNA and HCT116 with this short hairpin RNA were more resistant to cisplatin treatment. Finally, cells expressing E1A or Rb short interfering RNA were resistant to RSK4-mediated senescence. These results support the concept that RSK4 may be an important tumor suppressor gene by modulating senescence induction and contributing to cell proliferation control in colon carcinogenesis and renal cell carcinomas.

7. *EGFR/KRAS/BRAF* Mutations in Primary Lung Adenocarcinomas and Corresponding Locoregional Lymph Node Metastases, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4554-4560

PURPOSE AND METHODS: The epidermal growth factor receptor (EGFR) and its downstream factors KRAS and BRAF are mutated with different frequencies in non-small cell lung cancer and mutations predict clinical response to EGFR inhibitors. The present study compared the mutational status of EGFR, KRAS, and BRAF in primary tumors with the one in corresponding lymph node metastases. Direct bidirectional sequencing of EGFR gene exons 18 to 21, KRAS gene codons 12/13 and 61 to 68, and BRAF exon 15 was done on 96 paired samples of primary lung adenocarcinomas and corresponding locoregional lymph node metastases. In addition, comparative genomic hybridization analyses in two pairs of corresponding primary and metastatic tumor samples with discordant EGFR mutation status were done.

RESULTS AND CONCLUSION: Mutations in EGFR, KRAS, and BRAF were observed in 7 (7%), 36 (38%), and 2 (2%) patients, respectively. Interestingly, KRAS mutations were observed in two patients with an EGFR mutation. Mutations in primary tumors and lymph node metastases were identical in 1 of 7 (14%) patients in case of EGFR and 11 of 36 (31%) patients in case of KRAS. One patient harbored different KRAS mutations in primary and corresponding metastatic tumors. Comparative genomic hybridization analysis revealed similar patterns of chromosomal changes, strongly supporting a common clonal origin of primary tumors and metastases. The possibility of differences in the mutational status of EGFR, KRAS, BRAF between primary tumors and corresponding lymph node metastases should be considered whenever these mutations are used for the selection of patients for EGFR-directed tyrosine kinase inhibitor therapy.

8. High Levels of Circulating VEGFR2⁺ Bone Marrow-Derived Progenitor Cells Correlate with Metastatic Disease in Patients with Pediatric Solid Malignancies, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4561-4571

PURPOSE AND METHODS: Pediatric solid malignancies display important angiogenic potential, and blocking tumor angiogenesis represents a new therapeutic approach for these patients. Recent studies have evidenced rare circulating cells with endothelial features contributing to tumor neovascularization and have shown the pivotal role of bone marrow-derived (BMD) progenitor cells in metastatic disease progression. We measured these cells in patients with pediatric solid malignancies as a prerequisite to clinical trials with antiangiogenic therapy. Peripheral blood was drawn from 45 patients with localized (n = 23) or metastatic (n = 22) disease, and 20 healthy subjects. Subsets of circulating vascular endothelial growth factor receptor (VEGFR)2⁺-BMD progenitor cells, defined as CD45⁻CD34⁺VEGFR2(KDR)⁺7AAD⁻ and CD45^{dim}CD34⁺VEGFR2⁺7AAD⁻ events, were measured in progenitor-enriched fractions by flow cytometry. Mature circulating endothelial cells (CEC) were measured in whole blood as CD31⁺CD146⁺CD45⁻7AAD⁻ viable events. Data were correlated with VEGF and sVEGFR2 plasma levels.

RESULTS AND CONCLUSION: The CD45⁻CD34⁺VEGFR2(KDR)⁺7AAD⁻ subset represented <0.003% of circulating BMD progenitor cells (≤ 0.05 cells/mL). However, the median level (range) of the CD45^{dim}CD34⁺VEGFR2⁺7AAD⁻ subset was higher in patients compared with healthy subjects, 1.5% (0%-10.3%) versus 0.3% (0%-1.6%) of circulating BMD progenitors (P < 0.0001), and differed significantly between patients with localized and metastatic disease, 0.7% (0%-8.6%) versus 2.9% (0.6%-10.3%) of circulating BMD progenitors (P < 0.001). Median CEC value was 7 cells/mL (0-152 cells/mL) and similar in all groups. Unlike VEGFR2⁺-BMD progenitors, neither CECs, VEGF, or sVEGFR2 plasma levels correlated with disease status. High levels of circulating VEGFR2⁺-BMD progenitor cells correlated with metastatic disease. Our study provides novel insights for angiogenesis mechanisms in pediatric solid malignancies for which antiangiogenic targeting of VEGFR2⁺-BMD progenitors could be of interest.

9. 15-Hydroxyprostaglandin Dehydrogenase Is Down-regulated in Gastric Cancer, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4572-4580

PURPOSE AND METHODS: We have investigated the expression and regulation of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in gastric cancer. Clinical gastric adenocarcinoma samples were analyzed by immunohistochemistry and quantitative real-time PCR for protein and mRNA expression of 15-PGDH and for methylation status of 15-PGDH promoter. The effects of interleukin-1 β (IL-1 β) and epigenetic mechanisms on 15-PGDH regulation were assessed in gastric cancer cell lines.

RESULTS AND CONCLUSION: In a gastric cancer cell line with a very low 15-PGDH expression (TMK-1), the 15-PGDH promoter was methylated and treatment with a demethylating agent 5-aza-2'-deoxycytidine restored 15-PGDH expression. In a cell line with a relatively high basal level of 15-PGDH (MKN-28), IL-1 β repressed expression of 15-PGDH mRNA and protein. This effect of IL-1 β was at least in part attributed to inhibition of 15-PGDH promoter activity. SiRNA-mediated knockdown of 15-PGDH resulted in strong increase of prostaglandin E₂ production in MKN-28 cells and increased cell growth of these cells by 31% in anchorage-independent conditions. In clinical gastric adenocarcinoma specimens, 15-PGDH mRNA levels were 5-fold lower in gastric cancer samples when compared with paired nonneoplastic tissues (n = 26) and 15-PGDH protein was lost in 65% of gastric adenocarcinomas (n = 210). 15-PGDH is down-regulated in gastric cancer, which could potentially lead to accelerated tumor progression. Importantly, our data indicate that a proinflammatory cytokine linked to gastric carcinogenesis, IL-1 β , suppresses 15-PGDH expression at least partially by inhibiting promoter activity of the 15-PGDH gene.

10. Relationship of CDX2 Loss with Molecular Features and Prognosis in Colorectal Cancer, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4665-4673

PURPOSE AND METHODS: The homeodomain transcription factor CDX2 is a relatively specific immunohistochemical marker for gastrointestinal carcinoma. However, no study has comprehensively examined the relationship between CDX2 expression in colon cancer and clinical, pathologic, prognostic, and molecular features, including microsatellite instability and CpG island methylator phenotype (CIMP). Utilizing 621 colorectal cancers with clinical outcome and molecular data, CDX2 loss was detected in 183 (29%) tumors by immunohistochemistry.

RESULTS AND CONCLUSION: In multivariate logistic regression analysis, CDX2 loss was associated with female gender [odds ratio (OR), 3.32; $P < 0.0001$], CIMP-high (OR, 4.42; $P = 0.0003$), high tumor grade (OR, 2.69; $P = 0.0085$), stage IV disease (OR, 2.03; $P = 0.019$), and inversely with LINE-1 hypomethylation (for a 30% decline; OR, 0.33; $P = 0.0031$), p53 expression (OR, 0.55; $P = 0.011$), and β -catenin activation (OR, 0.60; $P = 0.037$), but not with body mass index, tumor location, microsatellite instability, BRAF, KRAS, PIK3CA, p21, or cyclooxygenase-2. CDX2 loss was not independently associated with patient survival. However, the prognostic effect of CDX2 loss seemed to differ according to family history of colorectal cancer ($P_{\text{interaction}} = 0.0094$). CDX2 loss was associated with high overall mortality (multivariate hazard ratio, 2.40; 95% CI, 1.28-4.51) among patients with a family history of colorectal cancer; no such association was present (multivariate hazard ratio, 0.97; 95% CI, 0.66-1.41) among patients without a family history of colorectal cancer. CDX2 loss in colorectal cancer is independently associated with female gender, CIMP-high, high-level LINE-1 methylation, high tumor grade, and advanced stage. CDX2 loss may be associated with poor prognosis among patients with a family history of colorectal cancer.

11. SMAD4 Gene Mutations Are Associated with Poor Prognosis in Pancreatic Cancer, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4674-4679

PURPOSE AND METHODS: Recently, the majority of protein coding genes were sequenced in a collection of pancreatic cancers, providing an unprecedented opportunity to identify genetic markers of prognosis for patients with adenocarcinoma of the pancreas. We previously sequenced more than 750 million base pairs of DNA from 23,219 transcripts in a series of 24 adenocarcinomas of the pancreas. In addition, 39 genes that were mutated in more than one of these 24 cancers were sequenced in a separate panel of 90 well-characterized adenocarcinomas of the pancreas. Of these 114 patients, 89 underwent pancreaticoduodenectomy, and the somatic mutations in these cancers were correlated with patient outcome.

RESULTS AND CONCLUSION: When adjusted for age, lymph node status, margin status, and tumor size, SMAD4 gene inactivation was significantly associated with shorter overall survival (hazard ratio, 1.92; 95% confidence interval, 1.20-3.05; $P = 0.006$). Patients with SMAD4 gene inactivation survived a median of 11.5 months, compared with 14.2 months for patients without SMAD4 inactivation. By contrast, mutations in CDKN2A or TP53 or the presence of multiple (≥ 4) mutations or homozygous deletions among the 39 most frequently mutated genes were not associated with survival. SMAD4 gene inactivation is associated with poorer prognosis in patients with surgically resected adenocarcinoma of the pancreas.

12. Vascular Endothelial Growth Factor Polymorphisms and Esophageal Cancer Prognosis, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4680-4685

PURPOSE AND METHODS: Vascular endothelial growth factor (VEGF) promotes angiogenesis and vascular permeability. The VEGF gene is polymorphic. We investigated the prognostic significance of three VEGF single nucleotide polymorphisms (SNP) in esophageal cancer. Three hundred sixty-one patients were genotyped for three VEGF SNPs (-460T/C, 405G/C, and 936C/T) using DNA extracted from prospectively collected blood samples. The association of each individual SNP, and haplotypes of the three SNPs, on overall survival (OS) was investigated.

RESULTS AND CONCLUSION: The variant allele of 936C/T was associated with improved OS compared with the wild-type genotype (log-rank $P < 0.001$). This association remained significant for OS after adjustments for age, gender, performance status, and disease stage [VEGF 936C/T: adjusted hazard ratio (AHR), 0.70; 95% confidence interval (95% CI), 0.49-0.99; $P = 0.04$; VEGF 936T/T: AHR, 0.11; 95% CI, 0.02-0.82; $P = 0.03$]. No independent associations were found for VEGF -460T/C and VEGF 405G/C. The CGC haplotype of the three VEGF SNPs (-460T/C, 405G/C, and 936C/T) combined was associated with reduced OS compared with all other patients (CGC/CGC: AHR, 1.51; 95% CI, 1.00-2.30; $P = 0.05$). VEGF 936C/T, and a haplotype of 460T/C, 405G/C, and 936C/T combined, has potential prognostic significance in esophageal cancer.

13. Human Leukocyte Antigen-G Protein Expression Is an Unfavorable Prognostic Predictor of Hepatocellular Carcinoma following Curative Resection, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4686-4693

PURPOSE AND METHODS: Human leukocyte antigen-G (HLA-G) is a tumor-associated immunosuppressive molecule involved in tumor escape mechanisms. The aim of this study is to elucidate its prognostic significance in hepatocellular carcinoma (HCC). Immunohistochemical staining of HLA-G expression as well as tumor-infiltrating FoxP3⁺ regulatory (Tregs) and CD8⁺ cytotoxic T cells was carried out on tissue microarrays containing 173 HCC tissue specimens. Membrane-bound HLA-G1 protein expression in five human HCC cell lines was detected by Western blot.

RESULTS AND CONCLUSION: HLA-G expression was associated with HCC prognosis, especially in early-stage diseases, with high expression independently associated with shortened overall survival ($P = 0.041$) and increased tumor recurrence ($P = 0.023$). HLA-G level was positively related to Tregs/CD8⁺ ratio and their combination served as a better prognosticator, patients having concurrent high levels of both variables at more than three times of risk of death and tumor relapse than those with concurrent low levels (both $P < 0.001$). In addition, HLA-G1 expression increased in a concordant manner with the increase of metastatic potential in human HCC cell lines. Overexpression of HLA-G protein in HCC was an independent indicator for poor outcome especially in early-stage disease. The combination of HLA-G expression and Tregs/CD8⁺ ratio added the prognostic power to both variables, offering a possible strategy of tumor-stroma interaction-oriented cancer immunotherapy.

14. The Influence of Prostate Volume on Prostate-Specific Antigen Performance: Implications for the Prostate Cancer Prevention Trial Outcomes, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4694-4699

PURPOSE AND METHODS: Although showing a 25% reduction in the biopsy prevalence of cancer compared with placebo in the Prostate Cancer Prevention Trial, finasteride was associated with a higher prevalence of high-grade disease. This observation was driven by "for-cause" biopsies. We sought to understand how volume-dependent changes in prostate-specific antigen test performance characteristics may have contributed. A retrospective review was done on 1,304 men referred for initial biopsy with a prostate-specific antigen between 4 and 10 ng/mL or an abnormal digital rectal examination. Receiver-operator curves and positive predictive values were ascertained for prostate-specific antigen stratified by diagnosis and prostate volume.

RESULTS AND CONCLUSION: The performance of prostate-specific antigen changed for any and high-grade (Gleason, $\geq 3 + 4$) cancer in a volume-specific manner. For any cancer, the area under the curve (AUC) decreased from 0.758 to 0.629 to 0.520 as prostate volume increased (<30, 30-50, >50 cm³, respectively). For high-grade cancer, a similar trend was shown (AUC, 0.712, 0.639, and 0.497, respectively). The positive predictive value of a prostate-specific antigen of ≥ 4 ng/mL was also affected by prostate volume. Trends for Gleason ≤ 6 decreased as prostate volume increased (positive predictive value for <30 cm³, 25.0%; positive predictive value for 30-50 cm³, 23.8%; and positive predictive value for >50 cm³, 17.3%). A more significant trend was seen for high-grade cancer (positive predictive value for <30 cm³, 39.0%; positive predictive value for 30-50 cm³, 22.3%; and positive predictive value for >50 cm³, 10.7%). Decreases in prostate volume over time and the resultant change in prostate-specific antigen performance characteristics may have contributed a bias toward the detection of high-grade disease in the finasteride arm of the Prostate Cancer Prevention Trial.

15. Color Fluorescence Ratio for Detection of Bronchial Dysplasia and Carcinoma In situ, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4700-4705

PURPOSE AND METHODS: Autofluorescence bronchoscopy is more sensitive than conventional bronchoscopy for detecting early airway mucosal lesions. Decreased specificity can lead to excessive biopsy and increased procedural time. Onco-LIFE, a device that combines fluorescence and reflectance imaging, allows numeric representation by expressing red-to-green ratio (R/G ratio) within the region of interest. The aim of the study was to determine if color fluorescence ratio (R/G ratio) added to autofluorescence bronchoscopy could provide an objective means to guide biopsy. Subjects at risk for lung cancer were recruited at two centers: VU University Medical Centre (Amsterdam) and BC Cancer Agency (Canada). R/G ratio for each site appearing normal or abnormal was measured before biopsy. R/G ratios were correlated with pathology, and a receiver operating characteristic curve of R/G ratio for high-grade and moderate dysplasia was done. Following analysis of the training data set obtained from two centers, a prospective validation study was done.

RESULTS AND CONCLUSION: Three thousand three hundred sixty-two adequate biopsies from 738 subjects with their corresponding R/G ratios were analyzed. R/G ratio 0.54 conferred 85% sensitivity and 80% specificity for the detection of high-grade and moderate dysplasia, area under the curve was 0.90, and 95% confidence interval was 0.88 to 0.92. In another 70 different sites that were assessed, measurements of agreement of R/G ratios with visual scores and pathology were 0.66 ($P < 0.0001$) and 0.61 ($P < 0.0001$), respectively. R/G ratio combined with visual score improved specificity to 88% (95% confidence interval, 0.73-0.96) for high-grade and moderate dysplasia. Color fluorescence ratio can objectively guide the bronchoscopist in selecting sites for biopsy with good pathologic correlation.

16. Prevalence of TMPRSS2-ERG Fusion Prostate Cancer among Men Undergoing Prostate Biopsy in the United States, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4706-4711

PURPOSE AND METHODS: Fusion of the TMPRSS2 prostate-specific gene with the ERG transcription factor is a putatively oncogenic gene rearrangement that is commonly found in prostate cancer tissue from men undergoing prostatectomy. However, the prevalence of the fusion was less common in samples of transurethral resection of the prostate from a Swedish cohort of patients with incidental prostate cancer followed by watchful waiting, raising the question as to whether the high prevalence in prostatectomy specimens reflects selection bias. We sought to determine the prevalence of TMPRSS2-ERG gene fusion among prostate-specific antigen-screened men undergoing prostate biopsy in the United States. We studied 140 prostate biopsies from the same number of patients for TMPRSS2-ERG fusion status with a fluorescent in situ hybridization assay. One hundred and thirty-four samples (100 cancer and 34 benign) were assessable.

RESULTS AND CONCLUSION: ERG gene rearrangement was detected in 46% of prostate biopsies that were found to have prostate cancer and in 0% of benign prostate biopsies ($P < 0.0001$). Evaluation of morphologic features showed that cribriform growth, blue-tinged mucin, macronucleoli, and collagenous micronodules were significantly more frequent in TMPRSS2-ERG fusion-positive prostate cancer biopsies than gene fusion-negative prostate cancer biopsies ($P \leq 0.04$). No significant association with Gleason score was detected. In addition, non-Caucasian patients were less likely to have positive fusion status ($P = 0.02$). This is the first prospective North American multicenter study to characterize TMPRSS2-ERG prostate cancer prevalence in a cohort of patients undergoing needle biopsy irrespective of whether or not they subsequently undergo prostatectomy. Our results show that this gene rearrangement is common among North American men who have prostate cancer on biopsy, is absent in benign prostate biopsy, and is associated with specific morphologic features. These findings indicate a need for prospective studies to evaluate the relationship of TMPRSS2-ERG rearrangement with clinical course of screening-detected prostate cancer in North American men, and a need for the development of noninvasive screening tests to detect TMPRSS2-ERG rearrangement.

17. Imaging Biomarkers Predict Response to Anti-HER2 (ErbB2) Therapy in Preclinical Models of Breast Cancer, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4712-4721

PURPOSE AND METHODS: To evaluate noninvasive imaging methods as predictive biomarkers of response to trastuzumab in mouse models of HER2-overexpressing breast cancer. The correlation between tumor regression and molecular imaging of apoptosis, glucose metabolism, and cellular proliferation was evaluated longitudinally in responding and nonresponding tumor-bearing cohorts. Mammary tumors from MMTV/HER2 transgenic female mice were transplanted into syngeneic female mice. BT474 human breast carcinoma cell line xenografts were grown in athymic nude mice. Tumor cell apoptosis (NIR700-Annexin V accumulation), glucose metabolism [2-deoxy-2- ^{18}F fluoro-D-glucose positron emission tomography (^{18}F FDG-PET)], and proliferation [3'- ^{18}F fluoro-3'-deoxythymidine-PET (^{18}F FLT-PET)] were evaluated throughout a biweekly trastuzumab regimen. Imaging metrics were validated by direct measurement of tumor size and immunohistochemical analysis of cleaved caspase-3, phosphorylated AKT, and Ki67.

RESULTS AND CONCLUSION: NIR700-Annexin V accumulated significantly in trastuzumab-treated MMTV/HER2 and BT474 tumors that ultimately regressed but not in nonresponding or vehicle-treated tumors. Uptake of ^{18}F FDG was not affected by trastuzumab treatment in MMTV/HER2 or BT474 tumors. ^{18}F FLT-PET imaging predicted trastuzumab response in BT474 tumors but not in MMTV/HER2 tumors, which exhibited modest uptake of ^{18}F FLT. Close agreement was observed between imaging metrics and immunohistochemical analysis. Molecular imaging of apoptosis accurately predicts trastuzumab-induced regression of HER2⁺ tumors and may warrant clinical exploration to predict early response to neoadjuvant trastuzumab. Trastuzumab does not seem to alter glucose metabolism substantially enough to afford ^{18}F FDG-PET significant predictive value in this setting. Although promising in one preclinical model, further studies are required to determine the overall value of ^{18}F FLT-PET as a biomarker of response to trastuzumab in HER2⁺ breast cancer.

18. Receptor-Targeted Nanoparticles for In vivo Imaging of Breast Cancer, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4722-4732

PURPOSE AND METHODS: Cell-surface receptor-targeted magnetic iron oxide nanoparticles provide molecular magnetic resonance imaging contrast agents for improving specificity of the detection of human cancer. The present study reports the development of a novel targeted iron oxide nanoparticle using a recombinant peptide containing the amino-terminal fragment of urokinase-type plasminogen activator (uPA) conjugated to magnetic iron oxide nanoparticles amino-terminal fragment conjugated-iron oxide (ATF-IO). This nanoparticle targets uPA receptor, which is overexpressed in breast cancer tissues.

RESULTS AND CONCLUSION: ATF-IO nanoparticles are able to specifically bind to and be internalized by uPA receptor-expressing tumor cells. Systemic delivery of ATF-IO nanoparticles into mice bearing s.c. and i.p. mammary tumors leads to the accumulation of the particles in tumors, generating a strong magnetic resonance imaging contrast detectable by a clinical magnetic resonance imaging scanner at a field strength of 3 tesla. Target specificity of ATF-IO nanoparticles showed by in vivo magnetic resonance imaging is further confirmed by near-IR fluorescence imaging of the mammary tumors using near-IR dye-labeled amino-terminal fragment peptides conjugated to iron oxide nanoparticles. Furthermore, mice administered ATF-IO nanoparticles exhibit lower uptake of the particles in the liver and spleen compared with those receiving nontargeted iron oxide nanoparticles. Our results suggest that uPA receptor-targeted ATF-IO nanoparticles have potential as molecularly targeted, dual modality imaging agents for in vivo imaging of breast cancer.

19. Identification of a New Panel of Serum Autoantibodies Associated with the Presence of In situ Carcinoma of the Breast in Younger Women, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4733-4741

PURPOSE AND METHODS: We examined the feasibility of using a panel of autoantibodies to multiple tumor-associated proteins as a method for early detection of breast cancer and, more particularly, carcinoma in situ (CIS). PPIA, PRDX2, and FKBP52 were identified as early-stage breast cancer autoantigens by proteomic approaches. The seroreactivity of a panel of antibodies consisting of these three antigens and two previously described autoantigens, HSP60 and MUC1, was tested on 235 samples (60 from primary breast cancer patients, 82 from CIS patients, and 93 from healthy controls) with the use of specific ELISAs. FKBP52, PPIA, and PRDX2 mRNA and protein expression levels were evaluated by reverse transcription-PCR and immunohistochemistry in early-stage breast tumors.

RESULTS AND CONCLUSION: Three of five autoantibodies, FKBP52, PPIA, and PRDX2, showed significantly increased reactivity in primary breast cancer and CIS compared with healthy controls. When combined, the five markers significantly discriminated primary breast cancer [receiver operating characteristic area under the curve, 0.73; 95% confidence interval (95% CI), 0.60-0.79] and CIS (receiver operating characteristic area under the curve, 0.80; 95% CI, 0.71-0.85) from healthy individuals. Importantly, the receiver operating characteristic-area under the curve value of the autoantibody panel was able to distinguish CIS, including high grades, from healthy controls in women under the age of 50 years (receiver operating characteristic area under the curve, 0.85; 95% CI, 0.61-0.92). Finally, only FKBP52 mRNA and protein levels were found to be increased in CIS and primary breast cancer compared with healthy breast tissue. This autoantibody assay against a panel of five antigens allows for an accurate discrimination between early-stage breast cancer, especially CIS, and healthy individuals. These results could be of interest in detecting early breast cancer as an aid to mammography, especially in women under the age of 50 years with aggressive cancers.

20. Differential Expression of Axl and Gas6 in Renal Cell Carcinoma Reflecting Tumor Advancement and Survival, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4742-4749

PURPOSE AND METHODS: Overexpression of the receptor tyrosine kinase Axl is implicated in several cancers. Therefore, we conducted this study to determine the expression of Axl and its ligand Gas6 in various renal cell carcinoma (RCC) types and in oncocytoma. Real-time quantitative reverse transcription-PCR was used to quantify tumor mRNA levels for Axl and Gas6 in a cohort (n = 221) of RCC patients. Serum levels of soluble sAxl and Gas6 proteins were measured using specific ELISA assays (n = 282). The presence of Axl protein in tumor tissue was evaluated by immunohistochemistry (n = 294). Results were correlated to tumor-associated variables, clinical biochemical tests, and patient survival.

RESULTS AND CONCLUSION: Tumor Axl mRNA levels correlated independently to survival when assessed against tumor stage and grade. In the study group, the median cancer-specific survival of all RCC patients during 307 months of follow-up was 55 months (confidence interval, ± 40.4). The 25% of patients with lowest tumor Axl mRNA levels had significantly better survival than the rest ($P = 0.0005$), with 70% of the patients still alive at the end of follow-up. In contrast, in patients with medium-high Axl mRNA, only 25% were alive at the end of follow-up. Tumor Gas6 mRNA levels correlated to survival, tumor-associated variables, and disease severity as did serum levels of soluble sAxl and Gas6 protein. However, no correlation between Axl protein in tumor tissue and survival was found. Axl and Gas6 expression in RCC are associated with tumor advancement and patient survival. In particular, low tumor Axl mRNA levels independently correlated with improved survival.

21. Clinical Relevance of a Pharmacogenetic Approach Using Multiple Candidate Genes to Predict Response and Resistance to Imatinib Therapy in Chronic Myeloid Leukemia, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4750-4758

PURPOSE AND METHODS: Imatinib resistance is major cause of imatinib mesylate (IM) treatment failure in chronic myeloid leukemia (CML) patients. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including amplification and overexpression of the BCR/ABL gene, the tyrosine kinase domain point mutations, and MDR1 gene overexpression. We investigated the impact of 16 single nucleotide polymorphisms (SNP) in five genes potentially associated with pharmacogenetics of IM, namely ABCB1, multidrug resistance 1; ABCG2, breast-cancer resistance protein; CYP3A5, cytochrome P450-3A5; SLC22A1, human organic cation transporter 1; and AGP, α 1-acid glycoprotein. The DNAs from peripheral blood samples in 229 patients were genotyped.

RESULTS AND CONCLUSION: The GG genotype in ABCG2 (rs2231137), AA genotype in CYP3A5 (rs776746), and advanced stage were significantly associated with poor response to IM especially for major or complete cytogenetic response, whereas the GG genotype at SLC22A1 (rs683369) and advanced stage correlated with high rate of loss of response or treatment failure to IM therapy. We showed that the treatment outcomes of imatinib therapy could be predicted using a novel, multiple candidate gene approach based on the pharmacogenetics of IM.

22. Development of Tumor-Reactive T Cells After Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplant for Chronic Lymphocytic Leukemia, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4759-4768

PURPOSE AND METHODS: Allogeneic nonmyeloablative hematopoietic stem cell transplant (NM-HSCT) can result in durable remission of chronic lymphocytic leukemia (CLL). It is thought that the efficacy of NM-HSCT is mediated by recognition of tumor cells by T cells in the donor stem cell graft. We evaluated the development of CTLs specific for CLL after NM-HSCT to determine if their presence correlated with antitumor efficacy. Peripheral blood mononuclear cells obtained from 12 transplant recipients at intervals after NM-HSCT were stimulated in vitro with CLL cells. Polyclonal T-cell lines and CD8⁺ T-cell clones were derived from these cultures and evaluated for lysis of donor and recipient target cells including CLL. The presence and specificity of responses was correlated with clinical outcomes.

RESULTS AND CONCLUSION: Eight of the 12 patients achieved remission or a major antitumor response and all 8 developed CD8⁺ and CD4⁺ T cells specific for antigens expressed by CLL. A clonal analysis of the CD8⁺ T-cell response identified T cells specific for multiple minor histocompatibility (H) antigens expressed on CLL in six of the responding patients. A significant fraction of the CD8⁺ T-cell response in some patients was also directed against nonshared tumor-specific antigens. By contrast, CLL-reactive T cells were not detected in the four patients who had persistent CLL after NM-HSCT, despite the development of graft-versus-host disease. The development of a diverse T-cell response specific for minor H and tumor-associated antigens expressed by CLL predicts an effective graft-versus-leukemia response after NM-HSCT.

23. A Phase I Clinical Trial of Darinaparsin in Patients with Refractory Solid Tumors, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4769-4776

PURPOSE AND METHODS: Darinaparsin, an organic arsenic, targets essential cell survival pathways. We determined the dose-limiting toxicity (DLT) and maximum tolerated dose of darinaparsin in patients with advanced cancer. Patients with solid malignancies refractory to conventional therapies were treated with i.v. darinaparsin daily for 5 days every 4 weeks. The starting dose (78 mg/m²) escalated to 109, 153, 214, 300, 420, and 588 mg/m². A conventional "3 + 3" design was used.

RESULTS AND CONCLUSION: Forty patients (median age, 61.5 years; median number of prior therapies, 5) received therapy; 106 cycles were given (median, 2; range, 1-12). Twenty patients reported no drug-related toxicities. No DLTs were reported at a dose of 420 mg/m^2. At 588 mg/m^2 , two of four patients developed DLTs, including grade 3 altered mental status and ataxia. Of four patients treated at the de-escalated dose of 500 mg/m^2 , one developed similar toxicities. De-escalating the dose to 420 mg/m^2 ($n = 8$) resulted in two neurologic DLTs. Further de-escalation to 300 mg/m^2 ($n = 3$) resulted in no drug-related toxicities. Arsenic plasma levels peaked on treatment day 3, plateaued on day 5, and returned to baseline on day 7. Plasma levels varied within cohorts but increased with increasing doses. The median plasma arsenic half-life was 16.2 hours. Seven (17.5%) patients had stable disease for ≥ 4 months (median, 6; range, 4-11), including 4 of 17 with colorectal and 2 of 3 with renal cancer. The recommended dose for phase II trials is 300 mg/m^2 i.v. given daily for 5 days every 4 weeks.

24. Partially Matched Related Donor Transplantation Can Achieve Outcomes Comparable with Unrelated Donor Transplantation for Patients with Hematologic Malignancies, *Clinical Cancer Research*, July 15, 2009, Vol. 15, Issue 14 pp 4777-4783

PURPOSE AND METHODS: The study aimed to compare the outcomes of patients undergoing hematopoietic stem cell transplantation (HSCT) from partially matched related donors (PMRD) and unrelated donors (URD) for hematologic malignancies without the use of in vitro T cell depletion. HSCT was done on 297 consecutive patients from URDs ($n = 78$) and PMRDs ($n = 219$) during the same time period. Incidences of graft-versus-host disease (GVHD), relapse, nonrelapse mortality, overall survival, and leukemia-free survival between the PMRD and URD groups were compared.

RESULTS AND CONCLUSION: All patients achieved full engraftment. The cumulative incidences of grades II to IV acute GVHD in the PMRD and URD cohorts were 47% [95% confidence interval (95% CI), 33-62%] versus 31% (CI, 20-42%; $P = 0.033$), with a relative risk of 1.72 (95% CI, 1.01-2.94; $P = 0.046$). The incidence of chronic GVHD did not differ significantly between the two cohorts ($P = 0.17$). The 2-year incidences of nonrelapse mortality and relapse were 20% (CI, 15-26%) versus 18% (CI, 10-27%), with $P = 0.98$, and 12% (CI, 8-16%) versus 18% (CI, 10-27%), with $P = 0.12$, for the PMRD versus the URD cohort, respectively. The 4-year overall survival and leukemia-free survival were 74% (CI, 67-80%) versus 74% (CI, 62-85%), with $P = 0.98$, and 67% (CI, 59-75%) versus 61% (CI, 47-74%), with $P = 0.74$, respectively. Our comparisons show that every major end point, including relapse, nonrelapse mortality, overall survival, and leukemia-free survival, was comparable between the PMRD and the URD groups.

FROM JULY 16, 2009, VOLUME 361 NO 3 ISSUE OF *NEW ENGLAND JOURNAL OF MEDICINE*

1. Capsule Endoscopy versus Colonoscopy for the Detection of Polyps and Cancer, *New England Journal of Medicine*, July 16, 2009, Vol. 361 Issue 3, pp 264-270

PURPOSE AND METHODS: An ingestible capsule consisting of an endoscope equipped with a video camera at both ends was designed to explore the colon. This study compared capsule endoscopy with optical colonoscopy for the detection of colorectal polyps and cancer. We performed a prospective, multicenter study comparing capsule endoscopy with optical colonoscopy (the standard for comparison) in a cohort of patients with known or suspected colonic disease for the detection of colorectal polyps or cancer. Patients underwent an adapted colon preparation, and colon cleanliness was graded from poor to excellent. We computed the sensitivity and specificity of capsule endoscopy for polyps, advanced adenoma, and cancer.

RESULTS AND CONCLUSION: A total of 328 patients (mean age, 58.6 years) were included in the study. The capsule was excreted within 10 hours after ingestion and before the end of the lifetime of the battery in 92.8% of the patients. The sensitivity and specificity of capsule endoscopy for detecting polyps that were 6 mm in size or bigger were 64% (95% confidence interval [CI], 59 to 72) and 84% (95% CI, 81 to 87), respectively, and for detecting advanced adenoma, the sensitivity and specificity were 73% (95% CI, 61 to 83) and 79% (95% CI, 77 to 81), respectively. Of 19 cancers detected by colonoscopy, 14 were detected by capsule endoscopy (sensitivity, 74%; 95% CI, 52 to 88). For all lesions, the sensitivity of capsule endoscopy was higher in patients with good or excellent colon cleanliness than in those with fair or poor colon cleanliness. Mild-to-moderate adverse events were reported in 26 patients (7.9%) and were mostly related to the colon preparation. The use of capsule endoscopy of the colon allows visualization of the colonic mucosa in most patients, but its sensitivity for detecting colonic lesions is low as compared with the use of optical colonoscopy.

FROM JULY, 2009 VOLUME 15, ISSUE 7 OF NATURE MEDICINE

1. Outpacing Cancer, *Nature Medicine*, July, 2009, Vol. 15 Issue 7, pp 718-722

ABSTRACT: In the late 1990s the drug gefitinib became a new tool in treating the most common type of lung cancer, called non-small cell lung cancer. But doctors found that even with continued gefitinib treatment, some patients experienced a cancer relapse within a year. For the past several years, researchers have been working to uncover why these patients lost sensitivity to gefitinib and seeking how to overcome resistance to the drug. Kirsten Dorans reports on the strategies scientists are developing to outpace continually evolving cancer.

2. Tumor immunotherapy: making an immortal army, *Nature Medicine*, July, 2009, Vol. 15 Issue 7, pp 731-732

ABSTRACT: Manipulation of cell renewal pathways creates T memory stem cells that can generate a sustained and targeted immune response. These findings have broad implications for vaccine development and immunotherapy (pages 808–813).

3. Targeted depletion of lymphotoxin—expressing TH1 and TH17 cells inhibits autoimmune disease, *Nature Medicine*, July, 2009, Vol. 15 Issue 7, pp 766-773

ABSTRACT: Uncontrolled T helper type 1 (TH1) and TH17 cells are associated with autoimmune responses. We identify surface lymphotoxin- (LT-) as common to TH0, TH1 and TH17 cells and employ a unique strategy to target these subsets using a depleting monoclonal antibody (mAb) directed to surface LT-. Depleting LT—specific mAb inhibited T cell-mediated models of delayed-type hypersensitivity and experimental autoimmune encephalomyelitis. In collagen-induced arthritis (CIA), preventive and therapeutic administration of LT—specific mAb inhibited disease, and immunoablated T cells expressing interleukin-17 (IL-17), interferon- and tumor necrosis factor- (TNF-), whereas decoy lymphotoxin- receptor (LT-R) fusion protein had no effect. A mutation in the Fc tail, rendering the antibody incapable of Fc receptor binding and antibody-dependent cellular cytotoxicity activity, abolished all in vivo effects. Efficacy in CIA was preceded by a loss of rheumatoid-associated cytokines IL-6, IL-1 and TNF- within joints. These data indicate that depleting LT—expressing lymphocytes with LT—specific mAb may be beneficial in the treatment of autoimmune disease.

FROM JULY 16, 2009, VOLUME 114, ISSUE 3 OF BLOOD

1. Extranodal Marginal Zone Lymphoma of the Ocular Adnexa, *Blood*, July 16, 2009, Vol. 114, Issue 3, pp 501-510

Lymphomas of the ocular adnexa are a heterogeneous group of malignancies, composing approximately 1% to 2% of non-Hodgkin lymphomas (NHLs) and 8% of extranodal lymphomas. The most common subtype, accounting for up to 80% of cases of primary ocular adnexal lymphoma, is marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type. In the recent past, there have been significant advances in our understanding of the clinical characteristics, morphology and phenotype, etiology, pathogenesis, diagnosis, natural history, treatment approaches, outcome, and prognostic factors of this disease entity. Novel immunologic and molecular techniques have aided in the distinction between MALT lymphoma and other lymphoproliferative disorders and led to the identification of tissue markers of prognostic significance. Modern imaging modalities provide invaluable tools for accurate staging and treatment planning. Besides radiotherapy and chemotherapy, a variety of new treatment options have emerged in the management of patients with ocular adnexal MALT lymphoma, especially monoclonal antibody therapy and antibiotic therapy against *Chlamydia psittaci*, which has been associated with the pathogenesis of ocular adnexal lymphomas in some parts of the world. In this review, we present a state-of-the-art summary of ocular adnexal MALT lymphomas.

2. Impact of Risk Stratification on Outcome Among Patients with Multiple Myeloma Receiving Initial Therapy with Lenalidomide and Dexamethasone, *Blood*, July 16, 2009, Vol. 114, Issue 3, pp 518-521

The outcome of patients with multiple myeloma is dictated primarily by cytogenetic abnormalities and proliferative capacity of plasma cells. We studied the outcome after initial therapy with lenalidomide-dexamethasone among 100 newly diagnosed patients, risk-stratified by genetic abnormalities and plasma cell labeling index. A total of 16% had high-risk multiple myeloma, defined by the presence of hypodiploidy, del(13q) by metaphase cytogenetics, del(17p), IgH translocations [t(4;14), or t(14;16)] or plasma cell labeling index more than or equal to 3%. Response rates were 81% vs 89% in the high-risk and standard-risk groups, respectively. The median progression-free survival was shorter in the high-risk group (18.5 vs 36.5 months, $P < .001$), but overall survival was comparable. Because of unavailability of all tests for every patient, we separately analyzed 55 stringently classified patients, and the results were similar. In conclusion, high-risk patients achieve less durable responses with lenalidomide-dexamethasone compared with standard-risk patients; no significant differences in overall survival are apparent so far. These results need confirmation in larger, prospectively designed studies.

3. Influence of Cytogenetics in Patients with Relapsed or Refractory Multiple Myeloma Treated with Lenalidomide Plus Dexamethasone: Adverse Effect of Deletion 17p13, *Blood*, July 16, 2009, Vol. 114, Issue 3, pp 522-525

Although the combination of lenalidomide and dexamethasone is effective therapy for patients with relapsed/refractory multiple myeloma, the influence of high-risk cytogenetic abnormalities on outcomes is unknown. This subanalysis of a large, open-label study investigated the effects of the most common unfavorable cytogenetic abnormalities detected by fluorescence in situ hybridization, del(13q), t(4;14), and del(17p13), in 130 evaluable patients treated with this regimen. Whereas patients with either del(13q) or t(4;14) experienced a median time to progression and overall survival comparable with those without these cytogenetic abnormalities, patients with del(17p13) had a significantly worse outcome, with a median time to progression of 2.22 months (hazard ratio, 2.82; $P < .001$) and median overall survival of 4.67 months (hazard ratio, 3.23; $P < .001$). Improved therapeutic strategies are required for this subgroup of patients.

4. Gene Therapy with Human and Mouse T-Cell Receptors Mediates Cancer Regression and Targets Normal Tissues Expressing Cognate Antigen, *Blood*, July 16, 2009, Vol. 114, Issue 3, pp 535-546

Gene therapy of human cancer using genetically engineered lymphocytes is dependent on the identification of highly reactive T-cell receptors (TCRs) with antitumor activity. We immunized transgenic mice and also conducted high-throughput screening of human lymphocytes to generate TCRs highly reactive to melanoma/melanocyte antigens. Genes encoding these TCRs were engineered into retroviral vectors and used to transduce autologous peripheral lymphocytes administered to 36 patients with metastatic melanoma. Transduced patient lymphocytes were CD45RA⁻ and CD45RO⁺ after ex vivo expansion. After infusion, the persisting cells displayed a CD45RA⁺ and CD45RO⁻ phenotype. Gene-engineered cells persisted at high levels in the blood of all patients 1 month after treatment, responding patients with higher ex vivo antitumor reactivity than nonresponders. Objective cancer regressions were seen in 30% and 19% of patients who received the human or mouse TCR, respectively. However, patients exhibited destruction of normal melanocytes in the skin, eye, and ear, and sometimes required local steroid administration to treat uveitis and hearing loss. Thus, T cells expressing highly reactive TCRs mediate cancer regression in humans and target rare cognate-antigen-containing cells throughout the body, a finding with important implications for the gene therapy of cancer.

WEEKLY JOURNAL REVIEW IN STEM CELLS

FROM JULY 16, 2009, VOLUME 114, ISSUE 3 OF *BLOOD*

1. Correction of Murine Hemophilia A Following Nonmyeloablative Transplantation of Hematopoietic Stem Cells Engineered to Encode an Enhanced Human Factor VIII Variant Using a Safety-Augmented Retroviral Vector, *Blood*, July 16, 2009, Vol. 114, Issue 3, pp 526-534

Insertional mutagenesis by retroviral vectors is a major impediment to the clinical application of hematopoietic stem cell gene transfer for the treatment of hematologic disorders. We recently developed an insulated self-inactivating gammaretroviral vector, RMSinOFB, which uses a novel enhancer-blocking element that significantly decreases genotoxicity of retroviral integration. In this study, we used the RMSinOFB vector to evaluate the efficacy of a newly bioengineered factor VIII (fVIII) variant (efVIII)—containing a combination of A1 domain point mutations (L303E/F309S) and an extended partial B domain for improved secretion plus A2 domain mutations (R484A/R489A/P492A) for reduced immunogenicity—toward successful treatment of murine hemophilia A. In cell lines, efVIII was secreted at up to 6-fold higher levels than an L303E/F309S A1 domain-only fVIII variant (sfVIII_B). Most important, when compared with a conventional gammaretroviral vector expressing sfVIII_B, lower doses of RMSin-efVIII-OFB-transduced hematopoietic stem cells were needed to generate comparable curative fVIII levels in hemophilia A BALB/c mice after reduced-intensity total body irradiation or nonmyeloablative chemotherapy conditioning regimens. These data suggest that the safety-augmented RMSin-efVIII-OFB platform represents an encouraging step in the development of a clinically appropriate gene addition therapy for hemophilia A.

FROM JUNE 2009, VOLUME 27, ISSUE 6 OF STEM CELLS

1. SOX15 and SOX7 Differentially Regulate the Myogenic Program in P19 Cells, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1231-1243

ABSTRACT: In this study, we have identified novel roles for Sox15 and Sox7 as regulators of muscle precursor cell fate in P19 cells. To examine the role of Sox15 and Sox7 during skeletal myogenesis, we isolated populations of P19 cells with either gene stably integrated into the genome, termed P19[Sox15] and P19[Sox7]. Both SOX proteins were sufficient to upregulate the expression of the muscle precursor markers Pax3/7, Meox1, and Foxc1 in aggregated cells. In contrast to the P19[Sox7] cell lines, which subsequently differentiated into skeletal muscle, myogenesis failed to progress past the precursor stage in P19[Sox15] cell lines, shown by the lack of MyoD and myosin heavy chain (MHC) expression. P19[Sox15] clones showed elevated and sustained levels of the inhibitory factors Msx1 and Id1, which may account for the lack of myogenic progression in these cells. Stable expression of a Sox15 dominant-negative protein resulted in the loss of Pax3/7 and Meox1 transcripts, as well as myogenic regulatory factor (MRF) and MHC expression. These results suggest that Sox15, or genes that are bound by Sox15, are necessary and sufficient for the acquisition of the muscle precursor cell fate. On the other hand, knockdown of endogenous Sox15 caused a decrease in Pax3 and Meox1, but not MRF expression, suggesting that other factors can compensate in the absence of Sox15. Taken together, these results show that both Sox7 and Sox15 are able to induce the early stages of myogenesis, but only Sox7 is sufficient to initiate the formation of fully differentiated skeletal myocytes.

2. Ly-1 Antibody Reactive Clone Is an Important Nucleolar Protein for Control of Self-Renewal and Differentiation in Embryonic Stem Cells, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1244-1254

ABSTRACT: Embryonic stem cells (ESCs) possess the capacity to self-renew and differentiate into all cell types of an organism. It is essential to understand how these properties are controlled for the potential usage of their derivatives in clinical settings and reprogramming of differentiated somatic cells. Although transcriptional factors, such as Oct4, Sox2, and Nanog, have been considered as a part of the core regulatory circuitry, a growing body of evidence suggests that additional factors exist and contribute to the control of ESC self-renewal and differentiation. Here, we report that Ly-1 antibody reactive clone (LYAR), a zinc finger nucleolar protein highly expressed in undifferentiated ESCs, plays a critical role in maintaining ESC identity. Its downregulation significantly reduces the rate of ESC growth and increases their apoptosis. Moreover, reduced expression of LYAR in ESCs impairs their differentiation capacity, failing to rapidly silence pluripotency markers and to activate differentiation genes upon differentiation. Mechanistically, LYAR forms a complex with another nucleolar protein, nucleolin, and prevents its self-cleavage, maintaining a normal steady-state level of nucleolin protein in undifferentiated ESCs. Interestingly, the downregulation of nucleolin is detrimental to the growth of ESCs and increases the rate of apoptosis, similarly to the knockdown of LYAR. Thus, our data emphasize the fact that other genes besides Oct4 and Nanog are uniquely required for ESC self-renewal and differentiation and demonstrate that LYAR functions to control the stability of nucleolin protein, which in turn is essential for maintaining the self-renewal of ESCs.

3. Epigenetic Reprogramming by Somatic Cell Nuclear Transfer in Primates, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1255-1264

ABSTRACT: We recently demonstrated that somatic cells from adult primates could be reprogrammed into a pluripotent state by somatic cell nuclear transfer. However, the low efficiency with donor cells from one monkey necessitated the need for large oocyte numbers. Here, we demonstrate nearly threefold higher blastocyst development and embryonic stem (ES) cell derivation rates with different nuclear donor cells. Two ES cell lines were isolated using adult female rhesus macaque skin fibroblasts as nuclear donors and oocytes retrieved from one female, following a single controlled ovarian stimulation. In addition to routine pluripotency tests involving in vitro and in vivo differentiation into various somatic cell types, primate ES cells derived from reprogrammed somatic cells were also capable of contributing to cells expressing markers of germ cells. Moreover, imprinted gene expression, methylation, telomere length, and X-inactivation analyses were consistent with accurate and extensive epigenetic reprogramming of somatic cells by oocyte-specific factors.

4. Alternative Translation of *OCT4* by an Internal Ribosome Entry Site and its Novel Function in Stress Response, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1265-1275

ABSTRACT: OCT4 is a pivotal transcription factor in maintaining the pluripotency and self-renewal capacities of embryonic stem (ES) cells. Human OCT4 can generate two isoforms by alternative splicing, termed OCT4A and OCT4B. OCT4A confers the stemness properties of ES cells, whereas the function of OCT4B is unknown. We present here the diverse protein products and a novel function of OCT4 gene. A single OCT4B mRNA can encode three isoforms by alternative translation initiation at AUG and CUG start codons, respectively. A putative internal ribosome entry site (IRES) has been identified in OCT4B mRNA accounting for the translation mechanism. The OCT4B-190 is upregulated under stress conditions and it may protect cell against apoptosis under stress. This work evokes the significance to distinguish the biological function of the protein products of OCT4. The OCT4 gene, by the regulation of alternative splicing and alternative translation initiation, may carry out more crucial roles in many biological events.

5. Stepwise Differentiation of Human Embryonic Stem Cells Promotes Tendon Regeneration by Secreting Fetal Tendon Matrix and Differentiation Factors, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1276-1287

ABSTRACT: Human embryonic stem cells (hESCs) are ideal seed cells for tissue regeneration, but no research has yet been reported concerning their potential for tendon regeneration. This study investigated the strategy and efficacy of using hESCs for tendon regeneration as well as the mechanism involved. hESCs were first induced to differentiate into mesenchymal stem cells (MSCs), which had the potential to differentiate into the three mesenchymal lineages and were positive for MSC surface markers. hESC-derived MSCs (hESC-MSCs) regenerated tendon tissues in both an in vitro tissue engineering model and an in vivo ectopic tendon regeneration model, as confirmed by the expression of tendon-specific genes and structure. In in-situ rat patellar tendon repair, tendon treated with hESC-MSCs had much better structural and mechanical properties than did controls. Furthermore, hESC-MSCs remained viable at the tendon wound site for at least 4 weeks and secreted human fetal tendon-specific matrix components and differentiation factors, which then activated the endogenous regeneration process in tendon. Moreover, no teratoma was found in any samples. These findings demonstrate a safe and practical strategy of applying ESCs for tendon regeneration and may assist in future strategies to treat tendon diseases.

6. Insights into Mesenchymal Stem Cell Aging: Involvement of Antioxidant Defense and Actin Cytoskeleton, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1288-1297

ABSTRACT: Progenitor cells such as mesenchymal stem cells (MSCs) have elicited great hopes for therapeutic augmentation of physiological regeneration processes, e.g., for bone fracture healing. However, regeneration potential decreases with age, which raises questions about the efficiency of autologous approaches in elderly patients. To elucidate the mechanisms and cellular consequences of aging, the functional and proteomic changes in MSCs derived from young and old Sprague-Dawley rats were studied concurrently. We demonstrate not only that MSC concentration in bone marrow declines with age but also that their function is altered, especially their migratory capacity and susceptibility toward senescence. High-resolution two-dimensional electrophoresis of the MSC proteome, under conditions of in vitro self-renewal as well as osteogenic stimulation, identified several age-dependent proteins, including members of the calponin protein family as well as galectin-3. Functional annotation clustering revealed that age-affected molecular functions are associated with cytoskeleton organization and antioxidant defense. These proteome screening results are supported by lower actin turnover and diminished antioxidant power in aged MSCs, respectively. Thus, we postulate two main reasons for the compromised cellular function of aged MSCs: (a) declined responsiveness to biological and mechanical signals due to a less dynamic actin cytoskeleton and (b) increased oxidative stress exposure favoring macromolecular damage and senescence. These results, along with the observed similar differentiation potentials, imply that MSC-based therapeutic approaches for the elderly should focus on attracting the cells to the site of injury and oxidative stress protection, rather than merely stimulating differentiation.

7. Epigenetic Landscaping During hESC Differentiation to Neural Cells, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1298-1308

ABSTRACT: The molecular mechanisms underlying pluripotency and lineage specification from embryonic stem cells (ESCs) are still largely unclear. To address the role of chromatin structure in maintenance of pluripotency in human ESCs (hESCs) and establishment of lineage commitment, we analyzed a panel of histone modifications at promoter sequences of genes involved in maintenance of pluripotency, self-renewal, and in early stages of differentiation. To understand the changes occurring at lineage-specific gene regulatory sequences, we have established an efficient purification system that permits the examination of two distinct populations of lineage committed cells; fluorescence activated cell sorted CD133⁺ CD45⁻CD34⁻ neural stem cells and β -III-tubulin⁺ putative neurons. Here we report the importance of other permissive marks supporting trimethylation of Lysine 4 H3 at the active stem cell promoters as well as poised bivalent and nonbivalent lineage-specific gene promoters in hESCs. Methylation of lysine 9 H3 was found to play a role in repression of pluripotency-associated and lineage-specific genes on differentiation. Moreover, presence of newly formed bivalent domains was observed at the neural progenitor stage. However, they differ significantly from the bivalent domains observed in hESCs, with a possible role of dimethylation of lysine 9 H3 in repressing the poised genes.

8. Fibroblast Growth Factor-2 Overexpression in Transplanted Neural Progenitors Promotes Perivascular Cluster Formation with a Neurogenic Potential, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1309-1317

ABSTRACT: Stem/progenitor cell-based therapies hold promises for repairing the damaged nervous system. However, the efficiency of these approaches for neuronal replacement remains very limited. A major challenge is to develop pretransplant cell manipulations that may promote the survival, engraftment, and differentiation of transplanted cells. Here, we investigated whether overexpression of fibroblast growth factor-2 (FGF-2) in grafted neural progenitors could improve their integration in the host tissue. We show that FGF-2-transduced progenitors grafted in the early postnatal rat cortex have the distinct tendency to associate with the vasculature and establish multiple proliferative clusters in the perivascular environment. The contact with vessels appears to be critical for maintaining progenitor cells in an undifferentiated and proliferative phenotype in the intact cortex. Strikingly, perivascular clusters of FGF-2 expressing cells seem to supply immature neurons in an ischemic environment. Our data provide evidence that engineering neural progenitors to overexpress FGF-2 may be a suitable strategy to improve the integration of grafted neural progenitor cells with the host vasculature thereby generating neurovascular clusters with a neurogenic potential for brain repair.

9. Identification of a Stroma-Mediated Wnt/ β -Catenin Signal Promoting Self-Renewal of Hematopoietic Stem Cells in the Stem Cell Niche, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1318-1329

ABSTRACT: With contrasting observations on the effects of β -catenin on hematopoietic stem cells (HSCs), the precise role of Wnt/ β -catenin signals on HSC regulation remains unclear. Here, we show a distinct mode of Wnt/ β -catenin signal that can regulate HSCs in a stroma-dependent manner. Stabilization of β -catenin in the bone marrow stromal cells promoted maintenance and self-renewal of HSCs in a contact-dependent manner, whereas direct stabilization in hematopoietic cells caused loss of HSCs. Interestingly, canonical Wnt receptors and β -catenin accumulation were predominantly enriched in the stromal rather than the hematopoietic compartment of bone marrows. Moreover, the active form of β -catenin accumulated selectively in the trabecular endosteum in "Wnt 3a-stimulated" or "irradiation-stressed," but not in "steady-state" marrows. Notably, notch ligands were induced in Wnt/ β -catenin activated bone marrow stroma and downstream notch signal activation was seen in the HSCs in contact with the activated stroma. Taken together, Wnt/ β -catenin activated stroma and their cross-talk with HSCs may function as a physiologically regulated microenvironmental cue for HSC self-renewal in the stem cell niche.

10. Activin A Is Essential for Neurogenesis Following Neurodegeneration, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1330-1346

ABSTRACT: It has long been proposed that excitotoxicity contributes to nerve cell death in neurodegenerative diseases. Activin A, a member of the transforming growth factor- β superfamily, is expressed by neurons following excitotoxicity. We show for the first time that this activin A expression is essential for neurogenesis to proceed following neurodegeneration. We found that intraventricular infusion of activin A increased the number of newborn neurons in the dentate gyrus, CA3, and CA1 layers of the normal adult hippocampus and also, following lipopolysaccharide administration, had a potent inhibitory effect on gliosis in vivo and on microglial proliferation in vivo and in vitro. Consistent with the role of activin A in regulating central nervous system inflammation and neurogenesis, intraventricular infusion of follistatin, an activin A antagonist, profoundly impaired neurogenesis and increased the number of microglia and reactive astrocytes following onset of kainic acid-induced neurodegeneration. These results show that inhibiting endogenous activin A is permissive for a potent underlying inflammatory response to neurodegeneration. We demonstrate that the anti-inflammatory actions of activin A account for its neurogenic effects following neurodegeneration because co-administration of nonsteroidal anti-inflammatory drugs reversed follistatin's inhibitory effects on neurogenesis in vivo. Our work indicates that activin A, perhaps working in conjunction with other transforming growth factor- β superfamily molecules, is essential for neurogenesis in the adult central nervous system following excitotoxic neurodegeneration and suggests that neurons can regulate regeneration by suppressing the inflammatory response, a finding with implications for understanding and treating acute and chronic neurodegenerative diseases.

11. cAMP Response Element Binding Protein Is Required for Mouse Neural Progenitor Cell Survival and Expansion, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1347-1357

ABSTRACT: Development of the mammalian brain relies on the coordinated expansion of neural cells in a relatively short time, spanning for a period of only a few days in mice. The molecular networks regulating neural cell birth and expansion, termed neurogenesis, are still unresolved, although many studies using genetically modified mice have revealed a growing number of genes that are involved in regulating these processes. The cAMP response element binding protein (CREB) lies at the hub of a diverse array of intracellular signaling pathways and is a major transcriptional regulator of numerous functions in adult neural cells, including learning and memory and neuronal survival. Recent studies have shown that activated CREB is highly expressed in immature dividing cells in adult mouse and zebrafish brains and that CREB regulates neural stem/progenitor cells (NSPCs) proliferation in embryonic zebrafish brain. Using genetically modified mice, we show that deletion of CREB, without the concomitant loss of the related compensating factor cAMP response element modifier, leads to defects in neural progenitor cell expansion and survival. Cultured primary CREB^{-/-} NSPCs exhibited decreased expression of several target genes important for neuronal survival and growth, including brain-derived neurotrophic factor and neural growth factor and showed that the survival and growth defect can be rescued by the addition of wild-type NSPC-conditioned medium. This is the first study showing a specific role for CREB in mammalian embryonic neurogenesis. This role appears to be mediated via the expression of factors important for NSPC survival and growth and suggests that CREB is an important signaling regulator within the developing neurogenic niche.

12. Integrin-Linked Kinase Is Required in Hypoxic Mesenchymal Stem Cells for Strengthening Cell Adhesion to Ischemic Myocardium, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1358-1365

ABSTRACT: Mesenchymal stem cells (MSCs) therapy has limitations due to the poor viability of MSCs after cell transplantation. Integrin-mediated adhesion is a prerequisite for cell survival. As a novel anti-death strategy to improve cell survival in the infarcted heart, MSCs were genetically modified to overexpress integrin-linked kinase (ILK). The survival rate of ILK-transfected MSCs (ILK-MSCs) was augmented by about 1.5-fold and the phosphorylation of ERK1/2 and Akt in ILK-MSCs were increased by about three and twofold, respectively. ILK-MSCs demonstrated an increase of twofold in the ratio of Bcl-2/Bax and inhibited caspase-3 activation, compared with hypoxic MSCs. The adhesion rate of ILK-MSCs also had a 32.2% increase on the cardiac fibroblast-derived three-dimensional matrix and ILK-MSCs showed higher retention by about fourfold compared to unmodified MSCs. Six animals per group were used for the in vivo experiments analyzed at 1 week after occlusion of the left coronary artery. ILK-MSC transplanted rats had a 12.0% \pm 3.1% smaller infarct size than MSC-treated rats after ligation of left anterior descending coronary artery. Transplantation of ILK-MSCs not only led to a 16.0% \pm 0.4% decrease in the fibrotic heart area, but also significantly reduced the apoptotic positive index by two-thirds when compared with ligation only. The mean microvessel count per field in the infarcted myocardium of ILK-MSCs group was increased relative to the sham group and MSCs group. In conclusion, the ILK gene transduction of MSCs further assisted cell survival and adhesion, and improved myocardial damage when compared with MSC only after transplantation.

13. Matrix Metalloproteinase 1 Is Necessary for the Migration of Human Bone Marrow-Derived Mesenchymal Stem Cells Toward Human Glioma, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1366-1375

ABSTRACT: Human mesenchymal stem cells (MSCs) have increasingly been used as cellular vectors for the delivery of therapeutic genes to tumors. However, the precise mechanism of mobilization remains poorly defined. In this study, MSCs that expressed similar cell surface markers and exhibited multilineage differentiation potentials were isolated from various donors. Interestingly, different MSC isolates displayed differential migration ability toward human glioma cells. We hypothesized that distinct molecular signals may be involved in the varied tumor tropisms exhibited by different MSC isolates. To test this hypothesis, gene expression profiles of tumor-trophic MSCs were compared with those of non-tumor-trophic MSCs. Among the various differentially regulated genes, matrix metalloproteinase one (MMP1) gene expression and its protein activities were enhanced by 27-fold and 21-fold, respectively, in highly migrating MSCs compared with poorly migrating MSCs. By contrast, there was no change in the transcriptional levels of other MMPs. Functional inactivation of MMP1 abrogated the migratory potential of MSCs toward glioma-conditioned medium. Conversely, the nonmigratory phenotype of poorly migrating MSC could be rescued in the presence of either recombinant MMP1 or conditioned medium from the highly migrating MSCs. Ectopic expression of MMP1 in these poorly migrating cells also rendered the cells responsive to the signaling cues from the glioma cells in vivo. However, blocking the interaction of MMP1 and its cognate receptor PAR1 effectively diminished the migratory ability of MSCs. Taken together, this study provides, for the first time, supporting evidence that MMP1 is critically involved in the migration capacity of MSCs, acting through the MMP1/PAR1 axis.

14. Reprogramming Retinal Pigment Epithelium to Differentiate Toward Retinal Neurons with Sox2, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1376-1387

ABSTRACT: Guiding non-neural, retinal pigment epithelium (RPE) to produce retinal neurons may offer a source of developing neurons for cell-replacement. Sox2 plays important roles in maintaining neural progenitor/stem cell properties and in converting fibroblasts into pluripotent stem cells. This study tests the possibility of using Sox2 to reprogram RPE to differentiate toward retinal neurons in vivo and in vitro. Expression of Sox2 in the chick retina was detected in progenitor cells, in cells at a discrete location in the layers of amacrine and ganglion cells, and in Müller glia. Overexpression of Sox2 in the developing eye resulted in hypopigmentation of the RPE. In the affected regions, expression of retinal ganglion cell markers became apparent in the RPE layer. In RPE cell culture, Sox2 promoted the expression of retinal ganglion and amacrine markers, and suppressed the expression of genes associated with RPE properties. Mechanistic investigation using the developing retina revealed a coexpression of Sox2 and basic fibroblast growth factor (bFGF), a growth factor commonly used in stem cell culture and capable of inducing RPE-to-retina transdifferentiation (or reprogramming) during early development. Similar patterns of changes in Sox2 expression and in bFGF expression were observed in atrophic retina and in injured retina. In RPE cell culture, Sox2 and bFGF mutually enhanced one another's expression. Upregulation of bFGF expression by Sox2 also occurred in the retina. These results suggest that Sox2 can initiate a reprogramming of RPE cells to differentiate toward retinal neurons and may engage bFGF during the process.

15. Epithelial Cells Derived from Human Embryonic Stem Cells Display P16^{INK4A} Senescence, Hypermotility, and Differentiation Properties Shared by Many P63⁺ Somatic Cell Types, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1388-1399

ABSTRACT: Human embryonic stem (hES) cells can generate cells expressing p63, K14, and involucrin, which have been proposed to be keratinocytes. Although these hES-derived, keratinocyte-like (hESderK) cells form epithelioid colonies when cultured in a fibroblast feeder system optimal for normal tissue-derived keratinocytes, they have a very short replicative lifespan unless engineered to express HPV16 E6E7. We report here that hESderK cells undergo senescence associated with p16^{INK4A} expression, unrelated to telomere status. Transduction to express bmi1, a repressor of the p16^{INK4A}/p14^{ARF} locus, conferred upon hESderK cells and keratinocytes a substantially extended lifespan. When exposed to transforming growth factor beta or to an incompletely processed form of Laminin-332, three lifespan-extended or immortalized hESderK lines that we studied became directionally hypermotile, a wound healing and invasion response previously characterized in keratinocytes. In organotypic culture, hESderK cells stratified and expressed involucrin and K10, as do epidermal keratinocytes in vivo. However, their growth requirements were less stringent than keratinocytes. We then extended the comparison to endoderm-derived, p63⁺/K14⁺ urothelial and tracheobronchial epithelial cells. Primary and immortalized lines of these cell types had growth requirements and hypermotility responses similar to keratinocytes and bmi1 expression facilitated their immortalization by engineering to express the catalytic subunit of telomerase (TERT). In organotypic culture, they stratified and exhibited squamous metaplasia, expressing involucrin and K10. Thus, hESderK cells proved to be distinct from all three normal p63⁺ cell types tested. These results indicate that hESderK cells cannot be identified conclusively as keratinocytes or even as ectodermal cells, but may represent an incomplete form of, or deviation from, normal p63⁺ lineage development.

16. Phenotypic and Functional Changes Induced in Hematopoietic Stem/Progenitor Cells After Gamma-Ray Radiation Exposure, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1400-1409

ABSTRACT: Ionizing radiation (IR) exposure causes rapid and acute bone marrow (BM) suppression that is reversible for nonlethal doses. Evidence is accumulating that IR can also provoke long-lasting residual hematopoietic injury. To better understand these effects, we analyzed phenotypic and functional changes in the stem/progenitor compartment of irradiated mice over a 10-week period. We found that hematopoietic stem cells (HSCs) identified by their repopulating ability continued to segregate within the Hoechst dye excluding "side population (SP)" early after IR exposure. However, transient phenotypic changes were observed within this cell population: Sca-1 (S) and c-Kit (K) expression levels were increased and severely reduced, respectively, with a concurrent increase in the proportion of SP^{SK} cells positive for established indicators of the presence of HSCs: CD150 and CD105. Ten weeks after IR exposure, expression of Sca-1 and c-Kit at the SP cell surface returned to control levels, and BM cellularity of irradiated mice was restored. However, the c-Kit⁺Sca-1⁺Lin^{-low} (KSL) stem/progenitor compartment displayed major phenotypic modifications, including an increase and a severe decrease in the frequencies of CD150⁺Fli2⁻ and CD150⁻Fli2⁺ cells, respectively. CD150⁺ KSL cells also showed impaired reconstituting ability, an increased tendency to apoptosis, and accrued DNA damage. Finally, 15 weeks after exposure, irradiated mice, but not age-matched controls, allowed engraftment and significant hematopoietic contribution from transplanted congenic HSCs without additional host conditioning. These results provide novel insight in our understanding of immediate and delayed IR-induced hematopoietic injury and highlight similarities between HSCs of young irradiated and old mice.

17. A Methodological Approach to Tracing Cell Lineage in Human Epithelial Tissues, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1410-1420

ABSTRACT: Methods for lineage tracing of stem cell progeny in human tissues are currently not available. We describe a technique for detecting the expansion of a single cell's progeny that contain clonal mitochondrial DNA (mtDNA) mutations affecting the expression of mtDNA-encoded cytochrome c oxidase (COX). Because such mutations take up to 40 years to become phenotypically apparent, we believe these clonal patches originate in stem cells. Dual-color enzyme histochemistry was used to identify COX-deficient cells, and mutations were confirmed by microdissection of single cells with polymerase chain reaction sequencing of the entire mtDNA genome. These techniques have been applied to human intestine, liver, pancreas, and skin. Our results suggest that the stem cell niche is located at the base of colonic crypts and above the Paneth cell region in the small intestine, in accord with dynamic cell kinetic studies in animals. In the pancreas, exocrine tissue progenitors appeared to be located in or close to interlobular ducts, and, in the liver, we propose that stem cells are located in the periportal region. In the skin, the origin of a basal cell carcinoma appeared to be from the outer root sheath of the hair follicle. We propose that this is a general method for detecting clonal cell populations from which the location of the niche can be inferred, also affording the generation of cell fate maps, all in human tissues. In addition, the technique allows analysis of the origin of human tumors from specific tissue sites.

18. Mesenchymal Stem Cell Transplantation Reverses Multiorgan Dysfunction in Systemic Lupus Erythematosus Mice and Humans, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1421-1432

ABSTRACT: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that, despite the advances in immunosuppressive medical therapies, remains potentially fatal in some patients, especially in treatment-refractory patients. Here, we reported that impairment of bone marrow mesenchymal stem cells (BMMSCs) and their associated osteoblastic niche deficiency contribute in part to the pathogenesis of SLE-like disease in MRL/lpr mice. Interestingly, allogenic BMMSC transplantation (MSCT) is capable of reconstructing the bone marrow osteoblastic niche and more effectively reverses multiorgan dysfunction when compared with medical immunosuppression with cyclophosphamide (CTX). At the cellular level, MSCT, not CTX treatment, was capable to induce osteoblastic niche reconstruction, possibly contributing to the recovery of regulatory T-cells and reestablishment of the immune homeostasis. On the basis of the promising clinical outcomes in SLE mice, we treated four CTX/glucocorticoid treatment-refractory SLE patients using allogenic MSCT and showed a stable 12–18 months disease remission in all treated patients. The patients benefited an amelioration of disease activity, improvement in serologic markers and renal function. These early evidences suggest that allogenic MSCT may be a feasible and safe salvage therapy in refractory SLE patients.

19. Wip1 Regulates the Generation of New Neural Cells in the Adult Olfactory Bulb through p53-Dependent Cell Cycle Control, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1433-1442

ABSTRACT: Continual generation of new neural cells from adult neural stem/progenitor cells (NPCs) is an important component of life-long brain plasticity. However, the intrinsic regulation of this process remains poorly defined. Here we report that Wip1 phosphatase, previously studied in oncogenesis, functions as a crucial physiological regulator in adult neural cell generation. Wip1 deficiency resulted in a 90% decrease in new cell formation in adult olfactory bulb, accompanied by aberrantly decreased NPC amplification, stem cell frequency, and self-renewal. At a cellular level, Wip1 knockout NPCs exhibit a prolonged cell cycle, an accumulation at G₂ to M phase transition, and enhanced p53 activity. Interestingly, the impaired M-phase entry and NPC amplification of Wip1-null mice can be reversed in Wip1/p53 double-null mice. Importantly, there is no difference in NPC amplification between p53-null and Wip1/p53 double-null mice. Our data demonstrate that Wip1 regulates the generation of new neural cells in adult olfactory bulb specifically through p53-dependent M-phase entry of the NPC cell cycle.

20. Analysis of Stem Cell Lineage Progression in the Neonatal Subventricular Zone Identifies EGFR⁺/NG2⁻ Cells as Transit-Amplifying Precursors, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1443-1454

ABSTRACT: In the adult subventricular zone (SVZ), astroglial stem cells generate transit-amplifying precursors (TAPs). Both stem cells and TAPs form clones in response to epidermal growth factor (EGF). However, in vivo, in the absence of sustained EGF receptor (EGFR) activation, TAPs divide a few times before differentiating into neuroblasts. The lack of suitable markers has hampered the analysis of stem cell lineage progression and associated functional changes in the neonatal germinal epithelium. Here we purified neuroblasts and clone-forming precursors from the neonatal SVZ using expression levels of EGFR and polysialylated neural cell adhesion molecule (PSANCAM). As in the adult SVZ, most neonatal clone-forming precursors did not express the neuroglia proteoglycan 2 (NG2) but displayed characteristics of TAPs, and only a subset exhibited antigenic characteristics of astroglial stem cells. Both precursors and neuroblasts were PSANCAM⁺; however, neuroblasts also expressed doublecortin and functional voltage-dependent Ca²⁺ channels. Neuroblasts and precursors had distinct outwardly rectifying K⁺ current densities and passive membrane properties, particularly in precursors contacting each other, because of the contribution of gap junction coupling. Confirming the hypothesis that most are TAPs, cell tracing in brain slices revealed that within 2 days the majority of EGFR⁺ cells had exited the cell cycle and differentiated into a progenitor displaying intermediate antigenic and functional properties between TAPs and neuroblasts. Thus, distinct functional and antigenic properties mark stem cell lineage progression in the neonatal SVZ.

21. Downregulation of APE1/Ref-1 Is Involved in the Senescence of Mesenchymal Stem Cells, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1455-1462

ABSTRACT: The senescence of human mesenchymal stem cells (hMSCs) causes disruption of tissue and organ maintenance, and is thus an obstacle to stem cell-based therapies for disease. Although some researchers have studied changes in the characteristics of hMSCs (decreases in differentiation ability and self-renewal), comparing young and old ages, the mechanisms of stem cell senescence have not yet been defined. In this study, we developed a growth curve for human bone marrow derived MSCs (hBMSCs) which changes into a hyperbolic state after passage number 7. Senescence associated β-galactosidase (SA β-gal) staining of hBMSCs showed 10% in passage 9 and 45% in passage 11. We detected an increase in endogenous superoxide levels during senescence that correlated with senescence markers (SA β-gal, hyperbolic growth curve). Interestingly, even though endogenous superoxide increased in a replicative senescence model, the expression of APE1/Ref-1, which is sensitive to intracellular redox state, decreased. These effects were confirmed in a stress-induced senescence model by exogenous treatment with H₂O₂. This change is related to the p53 activity that negatively regulates APE1/Ref-1. p21 expression levels, which represent p53 activity, were transiently increased in passage 9, meaning that they correlated with the expression of APE1/Ref-1. Overexpression of APE1/Ref-1 suppressed superoxide production and decreased SA β-gal in hBMSCs. In conclusion, intracellular superoxide accumulation appears to be the main cause of the senescence of hBMSCs, and overexpression of APE1/Ref-1 can rescue cells from the senescence phenotype. Maintaining characteristics of hBMSCs by regulating intracellular reactive oxygen species production can contribute to tissue regeneration and to improved cell therapy.

22. Human Gastrointestinal Neoplasia-Associated Myofibroblasts Can Develop from Bone Marrow-Derived Cells Following Allogeneic Stem Cell Transplantation, *Stem Cells*, June 2009, Vol. 27, Issue 6 pp 1463-1468

ABSTRACT: This study characterized the contribution of bone marrow-derived cells to human neoplasia and the perineoplastic stroma. The Australasian Bone Marrow Transplant Recipient Registry was used to identify solid organ neoplasia that developed in female recipients of male allogeneic stem cell transplants. Eighteen suitable cases were identified including several skin cancers, two gastric cancers, and one rectal adenoma. Light microscopy, fluorescence and chromogenic in situ hybridization, and immunohistochemistry were performed to determine the nature and origin of the neoplastic and stromal cells. In contrast to recent reports, donor-derived neoplastic cells were not detected. Bone marrow-derived neoplasia-associated myofibroblasts, however, were identified in the rectal adenoma and in a gastric cancer. Bone marrow-derived cells can generate myofibroblasts in the setting of human gastrointestinal neoplasia.

WEEKLY ONCOLOGY CONFERENCE REVIEW

Date	Name of the Conference	Location
July 31- Aug 4, 2009	13th World Conference on Lung Cancer	<i>San Francisco, CA</i>
Aug 2 - 7, 2009	Angiogenesis	<i>Newport, RI</i>
Aug 6 - 10, 2009	World Congress on Thyroid Cancer	<i>Toronto, Canada</i>
Sept 10 - 12, 2009	Living Fully With Cancer Conference	<i>Houston, TX</i>
Sept 13 - 18, 2009	Stem Cells and Cancer	<i>Les Diablerets, Switzerland</i>
Sept 20 - 24, 2009	ECCO 15 and 34th ESMO Multidisciplinary Congress	<i>Berlin, Germany</i>
Oct 1 - 4, 2009	Lynn Sage Breast Cancer Symposium	<i>Chicago, IL</i>
Oct 4 - 7, 2009	Scripps Cancer Center's 29th Annual Oncology Nurses Symposium	<i>San Diego, CA</i>
Oct 12 - 16, 2009	16th International Meeting of the European Society of Gynecological Oncology: EGO 2009	<i>Belgrade, Serbia</i>
Oct 21 - 24, 2009	3rd Thyroid Neoplasms Conference	<i>Sante Fe, NM</i>
Nov 11- 15, 2009	International Conference on Differentiation Therapy	<i>Chicago, IL</i>
Nov 11 - 14, 2009	Chemotherapy Foundation Symposium	<i>New York, NY</i>
Dec 10 - 13, 2009	The San Antonio Breast Cancer Symposium	<i>San Antonio, TX</i>

Source: Rodman & Renshaw, LLC

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Array BioPharma Inc.	ARRY	Market Outperform	\$3.21	\$154.43	\$10.00
CytRx Corp.	CYTR	Market Outperform	\$1.18	\$110.15	\$2.00
Genta Inc.	GNTA	Market Perform	\$0.01	\$NaN	NA
Oncolytics Biotech Inc.	ONCY	Market Outperform	\$1.59	\$78.49	\$2.00

RATING HISTORY

RATING SUMMARY

Distribution of Ratings Table					
Rating	Count	Percent	IB Serv./Past 12 Mos		
			Count	Percent	
Market Outperform(MO)	77	71.30%	11	14.29%	
Market Perform(MP)	24	22.20%	4	16.67%	
Market Underperform(MU)	0	0.00%	0	0.00%	
Under Review(UR)	7	6.50%	2	28.57%	
Total	108	100%	17	100%	

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