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## Contents



NATIONAL CANCER INSTITUTE
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DEPARTMENT OF HEALTH AND HUMAN SERVICES

■ CANCER AND CELL BIOLOGY

# Ongoing Chromosomal Instability in Cancer Cell Lines

Roschke AV, Stover K, Tonon G, Schäffer AA, Kirsch IR. Stable karyotypes in epithelial cancer cell lines despite high rates of ongoing structural and numerical chromosomal instability. *Neoplasia* 4: 19-31, 2002.

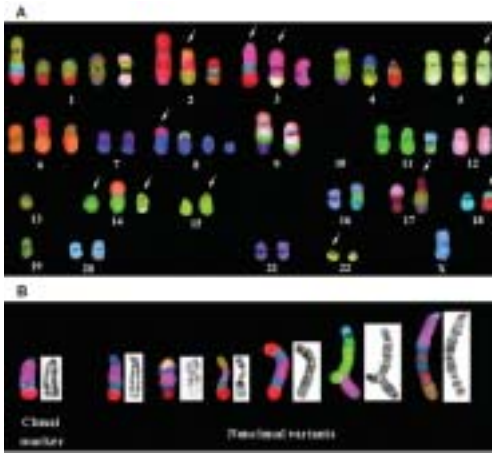

Figure 1. *A:* Spectral karyotype of the ovarian carcinoma cell line OVCAR-8 in display colors. Chromosomes frequently undergoing additional rearrangements are indicated with arrows. *B:* Examples of nonclonal variations of a clonal aberration. Nonclonal variants were collected from spectral karyotyping-analyzed cells of the OVCAR-8 parental cell line and subclones.

Most human tumors and tumor cell lines exhibit numerical and structural chromosomal abnormalities that are consequences of chromosomal instability. The level of instability cannot be determined by simply scoring the presence of genetic alterations in tumor cells, because these alterations may develop at different times at different rates. For instance, the presence of extensive chromosomal changes in human epithelial cancer cells may indicate that these cells had chromosomal instability during some phase of carcinogenesis, but it does not necessarily follow that this instability persists. In a previous study (Lengauer C et al. *Nature* 386: 623-7, 1997) contrasting microsatellite-unstable and microsatellite-stable but "chromosomally unstable" colorectal cancer cell lines, ongoing numerical chromosomal instability (resulting in losses or gains of chromosomes) was shown to persist only in microsatellite-stable cell lines, with a rate of $10^{-2}$ per chromosome per generation. The rate of ongoing structural chromosomal instability in human epithelial cancer cells remained to be elucidated. Our goals were to estimate this rate in several such cell lines and to compare the levels of persistent structural and numerical chromosomal instability in mismatch repair-deficient and -competent cell lines.

It is generally assumed that many cancer cell lines have relatively stable karyotypes over time. If this assumption is correct and can be experimentally validated, such validation would lead us to an additional question: is the rate of persisting chromosomal instability observed in cancer cell lines insufficient to produce karyotypic changes after many generations of growth in culture, or are there selective forces working against the incorporation of new karyotypic rearrangements into the signature karyotypes?

We performed comprehensive characterization of single-cell subclones of two colorectal (HCT-116 and HT-29) and two ovarian (SKOV-3 and OVCAR-8) cell lines and subsequently compared karyotypes from early and late passages. We chose these particular cell lines to represent not

only distinct tumor lineages but also mismatch repair-defective (HCT-116, SKOV-3) and mismatch repair-competent (HT-29, OVCAR-8) phenotypes. Characterization included 1) spectral karyotyping to detect structural rearrangements, 2) fluorescence *in situ* hybridization with a panel of centromeric probes to measure chromosome number changes, and 3) comparative genomic hybridization to analyze total genome dosage alterations. In addition, we used computer simulations to estimate the effects of the experimentally found rates of ongoing chromosomal instability on the karyotype of the cell population in the absence or presence of selection against new chromosomal aberrations.

We found that karyotypes of all four cell lines were aberrant. Not only did numerical chromosomal instability persist in cancer cells (the rate was approximately $10^{-3}$ per chromosome per generation), but ongoing structural instability was also present in the cancer cell lines (Figure 1 illustrates the instability observed in the OVCAR-8 cell line). We did not observe a profound difference between the cell lines that were mismatch repair defective or competent in the rates of numerical or structural instability.

Although ongoing chromosomal instability was demonstrated in all cell lines, the consensus karyotypes were relatively stable over many generations. To help define the parameters relating ongoing chromosomal instability and its effect on the karyotypes, we developed models and tested them by using computer simulation techniques. Simulations that assumed an equal chance of survival for all cells predicted a probability of seeing stable karyotypes that was inconsistent with our experimental findings. When the ability of cells to divide was decreased after the acquisition of new aberrant chromosomes, the simulations predicted relative stability of the karyotype over time. Thus, the data and simulations suggest that in steady-state conditions the emergence of new clonal chromosomal rearrangements occurs very slowly. If selection factors are applied, the chromosomal instability persisting in cancer cells provides karyotypic variants that have a selective advantage and might lead, for example, to development of drug resistance.

What could explain the karyotypic differences that distinguish these two groups of tumors (in general, mismatch repair-defective cells are near diploid and mismatch repair-competent cells are grossly aneuploid) if chromosomal instability is present in both of them? The HT-29 cell line is grossly aneuploid with many clonal chromosomal reconfigurations, while HCT-116 is near diploid with only a few aberrations. As noted, the rate of structural chromosomal instability was approximately the same in these cell lines, and the rate of numerical instability was less than two-fold higher in HT-29 than in HCT-116. Our computer simulations predicted that a two-fold difference in the rate of chromosomal instability would not explain this karyotypic difference. Although we have not definitively ruled out generation time or age of the cell line since its derivation as factors, circumstantial and epidemiological data suggest a different explanation. We favor a model in which, depending on the pathway of carcinogenesis, some tumors pass through a period of "cataclysmic" karyotypic destabilization with subsequent stabilization, while others (e.g., mismatch repair-defective malignancies) are able to bypass this period. This difference could lead to the observed karyotypic dichotomy.


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## Permissive Apoptosis-Resistance (PAR): A Two-step Model for Development of Resistance to Anticancer Drugs


Reinhold WC, Kouros-Mehr H, Kohn KW, Maunakea AK, Lababidi S, Roschke A, Stover K, Alexander J, Pantazis P, Miller L, Liu E, Kirsch IR, Urasaki Y, Pommier Y, and Weinstein JN. Apoptotic susceptibility of cancer cells selected for camptothecin resistance: gene expression profiling, functional analysis, and molecular interaction mapping. *Cancer Res* 63: 1000-11, 2003.


It's a depressingly familiar story in oncology: chemotherapy works well for a while. The tumor responds. The patient responds. But then the cancer recurs—having acquired resistance, not just to the drug used but to a broad range of drugs. The question is why? What molecular pathways or networks in the cell have changed to confer broad resistance?

To address that question, we compared the prostate cancer cell line DU145 with a variant (RC0.1) selected from it for resistance to a camptothecin drug by continuous exposure for many passages. DU145 is an androgen-independent, mismatch repair-deficient, microsatellite-unstable human cell line derived from metastatic disease. Camptothecins, several of which are currently in the clinic, specifically poison topoisomerase I (top1) by reversibly stabilizing DNA-top1 cleavable complexes (Pommier Y et al. *Drug Resist Updat* 2: 307-18, 1999). The sequence of top1 in RC0.1 contains a mutation (R364H) not present in DU145 that leaves the enzyme catalytically active but impervious to inhibition by camptothecins in biochemical assays. That mutation would suffice to explain RC0.1's camptothecin resistance.

We decided to ask, however, whether the RC0.1 had undergone other functionally important changes during its selection. To that end we used the NCI Microarray Facility's 1,648-clone cDNA array (Oncochip), which focuses on cancer-interesting genes, to compare the transcriptomes of DU145 and RC0.1. The arrays, which were run in quadruplicate under carefully controlled conditions, identified 181 genes that differed in transcript level by at least 1.5-fold between the two cell lines. Real-time reverse transcription PCR provided validation of the results for some of the genes.

When we classified the 181 changed genes by function, several categories were overrepresented. The largest of these was the "apoptosis-related" category (12 of 62 genes; $p = 0.038$ without multiple comparisons correction). Camptothecins, like other chemotherapies and irradiation, are known to induce apoptosis, so this finding was no surprise.

Given the number of changes in apoptosis-related transcripts, we decided to run functional assays to test the hypothesis

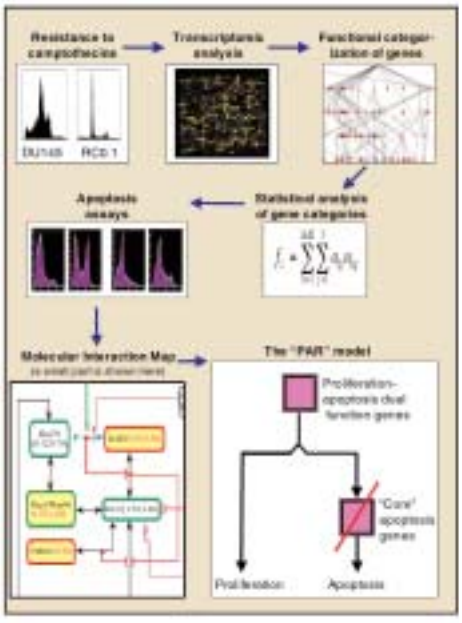


Figure 1. Logical and experimental flow leading to formulation of the permissive apoptosis-resistance (PAR) model for two-step development of drug resistance during selection by exposure to a drug. *Bottom right:* "Core" apoptosis genes change to inhibit apoptosis (red slash). Those changes permit dual function (proliferation-apoptosis) genes to change to favor proliferation.

that RC0.1 would show a generally reduced susceptibility to apoptosis. Using Annexin V and APO-BRDU flow cytometric assays to measure early and late stages of apoptosis, respectively, we found that the apoptotic response was indeed markedly reduced in RC0.1, not only to camptothecin (top1 poisoning) but also to cisplatin (bifunctional DNA damage), staurosporine (protein kinase inhibition), serum starvation (growth factor deprivation), ultraviolet radiation (DNA base damage), and ionizing radiation (DNA strand breakage). This pervasive functional difference would not have been expected on the basis of the known top1 mutation, but it fits well with the changes in apoptosis-related genes.

Then came the surprise and the paradox. Closer examination of the apoptosis-related genes revealed that more than half of them were changing in the "wrong" direction to explain RC0.1's resistance to apoptosis! To analyze that puzzling observation, we developed a detailed molecular interaction map (in the style of Kohn KW. *Chaos* 11: 84-97, 2001) of the salient pathways. The interaction map revealed a regularity: genes of the central apoptotic machinery (prominently including the Akt-PI3K, caspase, and Bcl-2 families) showed predominant expression changes in the direction "expected" for increased resistance to apoptosis in RC0.1. However, other sets of molecules associated with apoptosis but having dual proliferation-apoptosis functions—genes principally in the nuclear factor κB and transforming growth factor β pathways—had changed in the "wrong" direction with striking consistency. This pattern of observations led us to the two-stage "permissive apoptosis-resistance" (PAR) hypothesis and model to explain the development of resistance. According to this hypothesis, the first step is a block of the "core" apoptosis genes. That block permits the dual-function genes to change in a direction that confers a selective advantage by favoring proliferation. That change would not have been possible unless apoptosis of the cells had already been blocked. *c-MYC* and *E2F1* clearly fall into this category.

*Chemotherapy works well for a while. The tumor responds. The patient responds. But then the cancer recurs—having acquired resistance, not just to the drug used but to a broad range of drugs.*

The story described here reflects a synergy between "omic" and hypothesis-driven research (Weinstein JN et al. *Science* 275: 343-9, 1997; Weinstein JN. *Curr Opin Pharmacol* 2: 361-5, 2002) that will be required to elucidate the complexities of biological systems and to exploit the new understanding for biomedical purposes. We are currently following up the PAR hypothesis at the transcript level by assessing larger arrays of transcripts and at the proteomic level using "reverse-phase" protein lysate arrays. The biological reality is presumably much more complex than Figure 1 would suggest, but the PAR hypothesis at least serves as a starting point for further mechanistic inquiry. A thorough understanding of drug resistance and its development will help us realize the NCI's goal of eliminating suffering and death due to cancer.


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# Unexpected Mobility of Transcription Factors on Regulatory Elements

Becker M, Baumann C, John S, Walker DA, Vigneron M, McNally JG, and Hager GL. Dynamic behavior of transcription factors on a natural promoter in living cells. *EMBO Rep* 3: 1188-94, 2002.

The regulation of gene expression has been intensively studied since the first elucidation of the DNA | RNA | protein paradigm. Very early in this field it was established that transcription factors interact with regulatory regions in the general vicinity of the transcription initiation site for a given gene and that these factors somehow modulate the rate at which RNA polymerase is allowed to enter the promoter site and produce specific RNA transcripts. We now know that these regulatory proteins achieve their modulatory function through a large variety of mechanisms, including the extensive modification of the local chromatin domain.

We recently developed a method that permits direct visualization of gene expression in living cells. We first characterized a cell line in which a steroid receptor-regulated gene is amplified at one site on chromosome 4 in murine cell line 3134. An array containing approximately 200 copies of a perfect head-to-tail 9-kb repeat that includes the complete steroid-responsive MMTV promoter and reporter structure is inserted near the centromere (Figure 1). Each gene copy contains binding sites for six to eight receptors; thus, the array contains an aggregate capacity for approximately 1,000 receptors. We then introduced cell transcription factors tagged with the green fluorescence protein (GFP). Because the amplified gene array is localized at one site in the nucleus, it is possible to directly observe binding of GFP-labeled factors to the promoter repeated within the array. Thus, for the first time we could see the process of transcription activation in real time.

This system provided the opportunity to study, with photobleaching technologies, the movement of transcription-associated factors. In the most common approach, fluorescence recovery after photobleaching (FRAP), fluorescence proteins associated with a known target are rapidly bleached with an intense laser beam that can be focused on a small target within the nucleus and whose energy is tuned to the excitation wavelength of the fluorescence tag. If proteins subjected to such a bleaching protocol are stably bound to their target, the fluorescence signal will not recover and the target will remain bleached. If the factors are in dynamic equilibrium, however, the fluorescence will recover with kinetics determined by the proteins' rate of exchange.

Studies with factors that bind to the amplified gene revealed two general types of behavior. RNA Pol 2, which is responsible for polymerization of the RNA transcript, recovers slowly after the bleach pulse, requiring 13 minutes to regain the original fluorescence (Figure 2) (Becker M et al. *EMBO Rep* 3: 1188-94, 2002) because all of the elongating polymerases on each gene copy must complete their RNA chains and be released from the templates before they can be replaced by new, unbleached GFP-Pol 2 molecules. However, experiments with GFP-glucocorticoid receptor, which binds to the regulatory region and activates transcription from the gene, led to a highly unexpected finding (McNally JG et al. *Science* 287: 1262-65, 2000): the receptor is present only briefly, with a FRAP recovery period of 30 seconds. Thus, the receptor must exchange rapidly and constantly with the regulatory element.

These findings contrast markedly with the view of hormone action that has dominated endocrinology since the original characterization of ligand-binding nuclear receptors. In this classic view, receptors remain bound to their cognate genes essentially as long as hormone is present in the cell, and the receptors serve as platforms for the accumulation of large multi-protein complexes. These "co-activator" complexes in turn harbor the many activities required for gene activation. However, our findings indicate that the receptors are not "static" on the template but are moving rapidly on and off their regulatory elements. While initially controversial, recent findings with the estrogen receptor and a prolactin regulatory element (M. Mancini; reported at the 2003 Meeting of the Endocrine Society, June 19–22, Philadelphia, PA) now indicate that this rapid exchange is a common property of the steroid/nuclear receptor family.

It seems, therefore, that our view of receptor function is in need of major revision. We currently favor a model (Hager GL et al. *Curr Opin Genet Dev* 12: 137-41, 2002) in which ligand-activated receptors exist in the nucleoplasm in complexes with many different co-regulators. All of the complexes can interact transiently and randomly with hormone response elements specific for a given receptor. A productive interaction occurs only when the appropriate complex interacts at the regulatory site. As activities that alter the local chromatin domain are recruited to

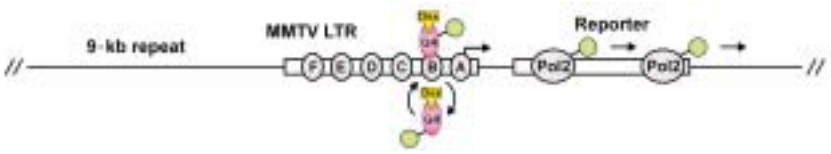


Figure 1. The target gene array contains 200 copies of a perfect head-to-tail 9-kb repeat that includes the complete promoter and reporter structure.

the element, the promoter is continuously modified and passes through a series of states. Which receptor-co-regulator complex will be productive at a given site is determined by the status of the promoter at the time of the receptor-template interaction.

Under our model, the nucleus is a highly dynamic environment. Most regulatory proteins are resident on their gene targets only for brief periods and continuously probe the response elements for current status. This model presents several attractive features. First, it accounts for the highly mobile nuclear behavior that has now been confirmed for many regulatory factors. Second, it offers a solution to the conundrum that many co-regulators in fact interact with the same domain of the primary recruiting factor (in our case the receptor). Finally, in many cases the co-activator harbors enzymatic activity (such as methylation and acetylation). Enzymes in general only interact transiently with the substrate; thus, a transcription factor that is an enzyme would interact only briefly with its target.

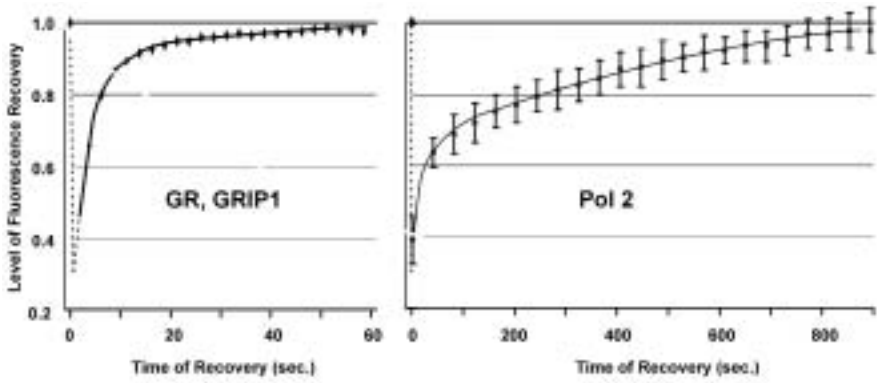


Figure 2. Fluorescence recovery after photobleaching analysis measures the mobility of transcription factors on the target gene. Both glucocorticoid receptor (GR) and its co-activator GRIP1 have very short resident times ($t_{1/2}$ = 5 seconds), whereas RNA Pol 2 requires 13 minutes for complete recovery.


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## ■ CLINICAL RESEARCH

# Clinical Trial Highlights: Focus on Prostate Cancer

### Protocol ID: 00-C-0080
### William Dahut, MD

### Phase III study of oral thalidomide versus placebo in patients with androgen-dependent stage IV nonmetastatic prostate cancer following limited hormonal ablation

Studies show that leuprolide and goserelin may fight prostate cancer by reducing the production of androgens. It is also believed that the antiangiogenesis drug thalidomide may stop prostate cancer growth by stopping blood flow to the tumor. This study will determine whether leuprolide or goserelin plus thalidomide are more effective than leuprolide or goserelin alone when treating patients with nonmetastatic prostate cancer. Patients must have progressive disease after definitive therapy with a prostate-specific antigen (PSA) level greater than 1. Patients who have rising PSA levels are randomly assigned to receive injections of either leuprolide or goserelin once a month, followed by daily oral doses of either thalidomide or a placebo until disease progression. After progression, patients receive leuprolide or goserelin for another 6 months. Patients who received placebos will then receive thalidomide, and patients who received thalidomide will then receive placebos. Patients are evaluated once a month and treatment continues for as long as benefit is shown.

### Protocol ID: 03-C-0157
### William Dahut, MD

### Phase II study of oral perifosine in patients with metastatic androgen-independent prostate cancer

Perifosine is one of a new class of anticancer drugs called alkylphospholipids (ALKS) that have shown broad anticancer activity and stabilized prostate cancer in some patients in preliminary clinical studies. Through this study, researchers will evaluate the safety and effectiveness of perifosine in treating metastatic prostate cancer that no longer responds to androgen ablation therapy. In phase I studies it was well tolerated with some gastrointestinal symptoms. Patients must have progressive disease with no more than one prior chemotherapy regimen. Patients receive perifosine orally for 3 weeks. Treatment repeats every 4 weeks in the absence of disease progression.

### Protocol ID: 02-C-0218
### William Dahut, MD

### Phase II pilot study of PSA vaccine with or without docetaxel in metastatic androgen-independent prostate cancer

Several clinical studies are evaluating the ability of vaccine therapy to make the body build an immune response to kill tumor cells. This study will compare the effectiveness of a PSA vaccine alone or in combination with docetaxel in treating patients who have metastatic prostate cancer. Researchers are attempting to determine if combining vaccine therapy with chemotherapy is more effective in killing tumor cells. The PSA vaccine used in this phase II study is composed of

four parts, including vaccinia virus plus human DNA that produces prostate-specific antigen (PSA), vaccinia virus plus human DNA that produces B7.1 (a protein that helps guide immune cells to their targets), fowlpox virus plus human DNA that produces PSA, and GM-CSF and IL-2, which enhance the ability of the immune system to kill tumor cells. Docetaxel is an approved treatment for breast, lung, and several other cancers. Patients must be HLA-A2 positive, have a rising PSA level, and show evidence of spreading disease.

Protocol ID: 02-C-0149
William Dahut, MD

### Phase I study of high-dose ketoconazole plus weekly docetaxel in patients with metastatic androgen-independent prostate cancer

High-dose ketoconazole and weekly docetaxel have both been shown to have activity against androgen-independent prostate cancer. This study tests the effectiveness of combining ketoconazole with docetaxel in the treatment of patients who have metastatic prostate cancer. Because ketoconazole may alter the metabolism of docetaxel, this study will also evaluate potential drug interactions and adverse events between these two agents. Patients will receive 30-minute infusions of docetaxel once a week for 3 out of 4 weeks. Beginning with the second course of treatment, patients will receive docetaxel once a week and begin ketoconazole continuous daily dosing. Treatment repeats every 4 weeks. Patients may remain in the study if their disease responds to treatment.

Protocol ID: 00-C-0154
James Gulley, MD, PhD

### Phase II study of radiotherapy with or without vaccine in patients with localized prostate cancer

This study will determine the ability of a vaccine to prevent the spread of localized prostate cancer following radiation therapy. The vaccine is intended to stimulate lymphocytes to target and attack cells containing prostate-specific antigen. Patients are randomized in a 2:1 ratio into one of two treatment arms: one will receive standard radiation therapy but no vaccine, the other will receive standard radiation therapy plus vaccinations before, during, and after radiation therapy in 28-day treatment cycles. Patients in both groups may receive hormone therapy, if indicated. The primary endpoint is to identify immunologic response as measured by *in vitro* analysis of patients' peripheral blood cells. The immune response of patients in both treatment arms will be analyzed at various times to determine whether the vaccination can affect a specific immune response, as well as whether radiotherapy has an effect on that immune response.

For more information about CCR clinical studies, contact the Clinical Studies Support Center at 1-800-NCI-1937.

## FROM THE DIRECTOR'S OFFICE

# C3D: An Innovative Tool for Managing Clinical Trials Data

The CCR oversees the development and implementation of approximately 200 intramural clinical trials that enroll almost 3,000 new patients annually. To answer the critical need for a large-scale, efficiently integrated Web-based information management system for clinical trials, the CCR entered into partnership with the NCI Center for Bioinformatics (NCICB) and the Oracle Corporation in early 2002 to develop the Cancer Central Clinical Database System (C3DS).

C3DS is being developed and deployed to the intramural community incrementally. The first of the system's five major components, the Cancer Central Clinical Database (C3D), was piloted in the Medical Oncology Research Unit (MOCRU) and is now active. C3D represents the system's primary data-capture module. Over the next 2 years, modules dedicated to registering and tracking participants in clinical trials, managing a central repository for human tissue specimens, warehousing clinical trial data, and tracking documentation related to protocol monitoring and regulatory compliance will become active. Once fully in place, the modules will function together as a seamlessly integrated whole.

The primary data-capture module, C3D, has been designed to satisfy the following requirements:

- Establish security procedures to protect patient confidentiality and maintain an audit trail as required by FDA regulations;
- Be integratable with NIH-wide and NCI information systems, most notably the Clinical Data Update System, the Clinical Trials Monitoring Service, the NCI's list of Common Data Elements, and the new NIH Clinical Center Clinical Research Information System (CRIS) scheduled for implementation in 2004; and
- Provide an array of informatics tools to support data standardization, validation, and quality assurance.

Based on a commercially available platform, C3D has been extensively customized to address the needs of intramural investigators and to facilitate the iterative flow of information essential to CCR translational research. When fully implemented, C3D will expedite the development and execution of clinical trials and provide a searchable, patient-centric database that will permit data mining across studies. The platform was engineered to be maximally flexible and has the capacity to evolve in response to user demands.

The C3D module now in operation consists of three components—Oracle Clinical, Remote Data Capture, and Oracle Discoverer. Oracle Clinical serves as the foundation of the system, supporting clinical trial definition, data capture,

multiple-site reporting, and standardization of data definitions and usage. Remote Data Capture provides a user-friendly interface that allows local and remote personnel to enter data, and electronically confirms source data verification. Oracle Discoverer gives authorized investigators real-time access to clinical data within and across clinical studies.

The heart of C3D is a library of standardized templates for collecting data required by specific clinical protocols, termed electronic Case Report Forms (eCRFs). The templates can be reused across multiple clinical studies; each study uses only those templates that are required and clinically appropriate. Once selected for a study, each template can be supplemented with additional questions. Study-specific choice lists will be created to help tailor forms. Phase-specific mandatory questions can be incorporated into individual studies. New eCRFs will be added to the standard templates archived in the library, automatically becoming candidates for reuse.

The template library will substantially accelerate the study implementation cycle, allowing many studies to be built within a 1- to 3-week time frame. At the time of the initial Institutional Review Board (IRB) filing, the clinical research team will identify data elements to be captured, select appropriate eCRF templates from the Web, and submit requests for modifications to chosen templates. The team will then review and test the templates with the objective of having the protocol database ready for implementation when the study receives IRB approval. Standardized templates will not only facilitate the building of protocol-specific databases, but also will facilitate data reporting, cross-coverage, and training. Education resources for the use and customization of C3D eCRFs, as well as other aspects of C3DS, are available on the system's website (http://ccrtrials.nci.nih.gov/CCR_trials/C3DS/C3D).

C3DS has been designed to be exportable to other cancer centers interested in using the CCR clinical trial business model. The system's exportability is a key element supporting the Cancer Bioinformatics Grid (caBIG) initiative being carried out by the NCICB under the directorship of Kenneth Buetow, PhD. The goal of caBIG is to develop an integrative biomedical informatics infrastructure with the capacity to support the cancer research community. The resulting database interoperability is expected to facilitate collaboration and cooperation among researchers in different institutions. The synergy made possible by a fully integrated national bioinformatics system will accelerate the pace of discovery and translation not only at the NCI, but also across the entire U.S. research spectrum.

For more information on caBIG, visit http://cabig.nci.nih.gov/caBIG/.

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Medical Director
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## ■ ADMINISTRATIVE LINKS

### A-76 Competitive Sourcing Program

Many NCI staff members have questions and concerns related to the A-76 Competitive Sourcing Program. The NIH Office of Management Assessment (OMA) has established a website to provide information about competitive sourcing activities being conducted at NIH in support of the President's Management Agenda's Competitive Sourcing Initiative. The Competitive Sourcing Initiative is intended "to achieve efficient and effective competition between public and private sources, [and] the Administration has committed itself to simplifying and improving the procedures for evaluating public and private sources, to better publicizing the activities subject to competition, and to ensuring senior-level agency attention to the promotion of competition." The website maintains an archive of all A-76 memorandums to NIH employees, definitions, related links, frequently asked questions (FAQs), and other resources. To learn more, go to http://a-76.nih.gov/.

### New Sponsored-Travel Procedures for Non-FTEs

The HHS-348 mechanism cannot be used for sponsored travel for non-FTE (full-time equivalent) persons. A non-FTE person is one who serves in some capacity while engaged in research- and science-related programs or activities for or at the NIH, but who is not appointed to duty. For example, an individual who receives a fellowship award (i.e., Visiting Fellow, IRTA, CRTA) is considered a non-FTE person. Special volunteers and guest researchers are also considered non-FTE persons.

A new NIH Manual Chapter, 1500-10 "Temporary Duty Travel and Relocation of Non-FTE Persons," was issued recently. Non-FTE sponsored-travel request packages should now consist of the following:

- Acceptance letter (Appendix 10);
- Certification checklist for sponsored travel of non-FTE persons (Appendix 10B);
- Invitation;
- Travel order.

All non-FTE sponsored-travel packages should be submitted to the employee's NCI Ethics servicing office for clearance 15 days or more in advance of travel. For more information, go to http://www1.od.nih.gov/oma/manualchapters/management/1500/10.html.

### Personal Digital Assistants (PDAs): Purchasing Moratorium Lifted

The HHS-wide moratorium on the purchase of PDAs has been lifted. The recommended HHS model is the RIM Blackberry 6710 (GPRS) due to its capability to support international use. NIH PDA-related policy, procedures, and purchasing information can be found at http://isdp.nih.gov/hardware/blackberry_acquire.asp.

## CORRECTION

We regret that the May 2003 issue contained an incorrect caption for the figure appearing in the article "Positional cloning of the Birt-Hogg-Dubé disease gene from a region of unique genome architecture" (Nickerson ML, Zbar B. *Frontiers in Science* May: 3-4, 2003). The figure is re-printed here with its correct caption.

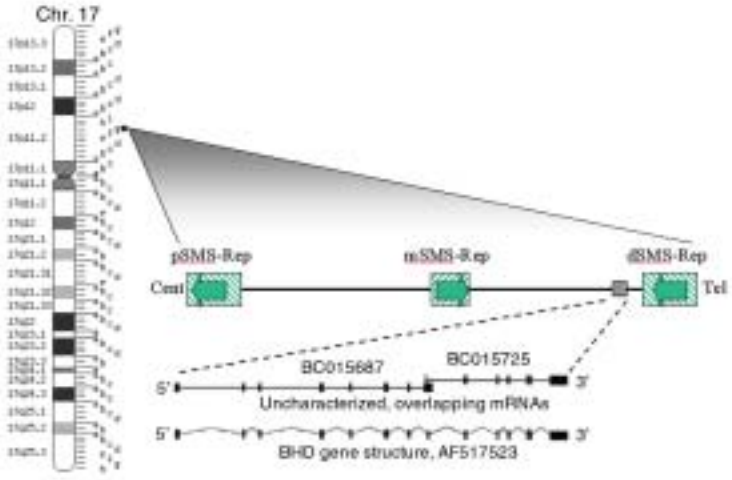


Figure 1. Location of the Birt-Hogg-Dubé (BHD) gene on chromosome 17. A 4-Mb region of 17p11.2 containing the proximal, middle, and distal Smith-Magenis syndrome repeats (pSMS-Rep, mSMS-Rep, and dSMS-Rep) is shown (above). SMS-Reps are approximately 200 kb each (hatched boxes). Green arrows indicate the orientation of the repeats relative to each other on the chromosome. They are about 98% identical due to recent duplication of the original repeat during primate evolution. Thirty genes and EST clusters, including splice variants, between the distal and middle SMS-Reps were sequenced before disease-associated mutations in the BHD gene were found (stippled box, not to scale). Proximal and distal SMS-Reps participate in nonallelic homologous recombination, which deletes the intervening genomic sequence, including BHD, to cause SMS. Genomic organization of BHD transcripts (Genbank accession numbers are listed) spans almost 25 kb of genome (below, not to scale). Cent, centromere; Tel, telomere.


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