

## Abstracts

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### **Biomarkers in the critical path to accelerate oncology drug development: Opportunities and road-blocks**

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The steps in oncology drug development in patients include: optimizing dose-schedule, predicting patients that will respond, detecting tumor responses rapidly for proof of concept trials, using surrogate endpoints for disease monitoring, assuring safety of drug therapy, and developing rational-based combination therapies. Biomarkers are pivotal in meeting each of these challenges. Examples of how Novartis has used biomarkers in drug development will be presented. These include: choosing the right patient by using mutations in target (c-Kit) to expand Glivec indications to GIST; validating that the drug hits the target by measuring phosphoS6 decreases as measure of mTOR inhibition, determining the optimal dose and schedule by measuring downstream signaling inhibition (phosphoAKT) and inhibition of proliferation and apoptosis in tumor tissue in POC trials; discovering rational based combination therapies; overcoming the mechanism of aromatase inhibitor resistance in breast cancer by targeting the compensatory pathway through the addition of a second Novartis compound; ensuring drug safety by identifying genotypes (UGT1A1) in metabolizing enzymes that predict Hyperbilirubinemia; and developing surrogate endpoints using major molecular responses using bcr-abl transcript levels for CML monitoring. A general strategy for using biomarkers in oncology drug development will be presented and includes: having a systematic biomarker plan for each new agent that is consistent, science-based and focused using common standards for assays and data; building a biomarker tool kit with analytical and clinical validated biomarker assays; building on clinical experience (positive and negative) and execution excellence involving a team effort (physicians, clinical staff, biomarker experts and

data management) and building a strong partnership between Novartis and its clinical investigators.

### **Mitochondrial mutations: Clonal evolution and functional consequences**

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We examined the pattern of mitochondrial DNA (mtDNA) mutation in bronchoscopically abnormal airway mucosal biopsies and matched tumors from two resected lung cancer patients. The airway mucosal biopsies were histopathologically diagnosed as normal but exhibited multiple clonal mtDNA mutations which were detectable in the corresponding tumors. One of the patients was operated twice for the removal of tumor from right upper and left lower lobe respectively within a span of two years. Both the tumors exhibited twenty identical mtDNA mutations suggesting identical clonal origin. Moreover, we identified clonal mitochondrial mutations in sputum from lung cancer patients and urine from bladder cancer patients but not controls. Our results support the continued prospective utilization of mtDNA mutation as a tool for cancer detection and monitoring.

### **Merging biomarker and biomeasure data into comprehensive estimates of disease risk**

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While the Holy Grail of cancer biomarkers is a single protein or other signature in a body fluid or other biospecimen that, if present indicates the presence of the index tumor and if absent that the tumor is not present, the likelihood of such a discovery is low. For all current biomarkers, the 'usual' approach to biomarker develop includes an analysis of performance characteristics (e.g., sensitivity, specificity, predictive values) and a decision on a 'cutpoint' value that segregates an 'abnormal' test from a 'normal' one.

The challenge with this approach is that it does not take into account prior probabilities of disease which can affect test performance. Not only may other risk factors (e.g., smoking status, parity, age) affect test performance and potential utility of the test but evaluation of a single biomarker in such a fashion precludes the incorporation of additional biomarkers that may improve the performance of the 'screening encounter' where the clinician makes a determination of disease risk followed by a decision for subsequent confirmatory testing.

Prostate cancer is the most common noncutaneous cancer in U.S. men with a 17% lifetime risk. Historically, PSA and digital rectal examination (DRE) have been used for the assessment of risk: If PSA is  $\geq 4.0$  ng/mL and/or if DRE is abnormal, a biopsy has historically been recommended. In the Prostate Cancer Prevention Trial (PCPT), we found that PSA is not dichotomous but is associated with disease risk. Taking the process further, we evaluated a panel of risk factors including age, PSA, family history of prostate cancer, race/ethnicity, DRE, and whether the person had a prior negative prostate biopsy for the assessment of disease risk. From these risk factors, *all of which had an independent predictive value for either prostate cancer and/or high grade prostate cancer*, we developed the PCPT Prostate Cancer Risk Calculator (available online). This risk calculator is increasingly becoming the standard of care for prostate cancer clinical assessment. It has now been validated in 3 additional independent cohorts.

Conclusions from this risk calculator have been multiple. First, age-adjusted PSA values have been found to increase the likelihood of detection of indolent can-

cer in young men while increasing the risk of missing aggressive cancer in older men. The use of DRE as normal/abnormal has been found to be ill-advised – for example, an abnormal DRE in a young man with a low PSA could have as much as an 8-fold lower risk of prostate cancer than a normal DRE in an older, higher-risk man whose PSA is 2.4 ng/mL (a 'normal' value by all current guidelines).

This risk assessment tool lends itself to the incorporation of new biomarkers, improving test sensitivity and specificity. We have recently incorporated PCA3 into the risk calculator, noting that the performance characteristics of the tool are improved. We encourage the use of this methodology in the development of new cancer screening tests.

### **Lessons learned in EDRN: The first eight years**

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As a member of the group that recommended the formation of the Early Detection Research Network (EDRN) and witnessed its launch in 2000, I am pleased to be involved in this workshop to review the lessons learned over the last eight years. More recently, I served on the panel invited to the progress the EDRN has made and the challenges it faces. The stated principal focus of the consortium in its recent report published in January is the creation of "validated biomarkers ready for large-scale clinical testing and eventual application." Despite the fact that eight years is a relatively short time for the de novo formation of a new consortium in a novel area of research, we can start to examine the EDRN's progress in the areas of structure, process, and outcome. With regard to structure, the consortium now includes more than 300 investigators and 40 private sector and academic institutions. As originally planned the membership includes scientists from divergent disciplines, including genomics, proteomics, metabolomics, bioinformatics, theoretical and applied statistics, clinical expertise, and public health. As an investigator driven consortium, it has brought together scientists with expertise in four major areas: biomarker discovery, marker validation, epidemiology/clinical study design, and statistics. Some of the early period of the consortium that incorporated such a widely divergent spectrum of disciplines and perspectives required formulation of new processes to move from the

traditional “horizontal” approach to biomarker investigation in which individual research groups developed biomarkers and validated them using their own home-grown definitions of “validation,” often with no mechanism to move along the continuum toward proof of benefit to the public. A process of “vertical” development of biomarkers was required, and the EDRN was the first to tackle this in the field. An important strategy was the development of five phases of biomarker development – a concept long used in drug development, but a true innovation in early detection biomarker research. But this is necessarily a work in progress, and the challenge of refining steps within the framework remains. Finally, the EDRN must ultimately be judged on outcomes. This is the hardest, but there is strong evidence of progress. Over 100 markers are in the EDRN pipeline, and there is an emerging collaboration between the EDRN investigators and the investigators of the Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial to conduct later phase studies of promising biomarkers identified in the EDRN. Has the process yielded early detection tools of proven benefit? Perhaps not, but eight years is a short time. It is often said that the development of a proven cancer drug takes about two decades. More importantly, the EDRN was conceived as a research engine with both an accelerator and a brake. The brake is important to prevent premature application of biomarkers for screening the healthy public. So a more difficult success to judge is the prevention of some of the mistakes of the past in the field of cancer screening. On balance, I submit that the EDRN has been a success in structure, process, as well as outcomes even if substantial challenges remain, and their remains much to be done in judging the ultimate outcomes.

### **Breast tumor heterogeneity and its clinical relevance**

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Breast cancer is a heterogeneous disease including multiple tumor subtypes associated with distinct clinical outcomes. Besides the high degree of inter-tumoral variability significant intra-tumoral heterogeneity also

exists that likely contribute to therapeutic resistance and recurrence. Understanding the molecular basis of breast tumor heterogeneity is key for the development of targeted cancer preventative and therapeutic interventions. Current models explaining inter and intra-tumoral diversity are the cancer stem cell and the clonal evolution hypotheses.

To characterize cells with stem-like characteristics from normal and neoplastic breast tissue, we determined the gene expression, epigenetic, and genetic profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Gene expression profiles were analyzed using SAGE (Serial Analysis of Gene Expression), genetic alterations were investigated using SNP (Single Nucleotide Polymorphism) arrays and FISH (Fluorescence In Situ Hybridization), and DNA methylation patterns were analyzed using MSDK (Methylation-Specific Digital Karyotyping).

The CD24+ more differentiated luminal epithelial and the CD44+ stem cell-like breast cancer cell populations from the same tumor were clonally related but not always identical and epigenetically distinct. A gene signature specific for CD44+ breast cancer cells was enriched for known stem cell markers and was associated with decreased overall and distant metastasis free survival in lymph node negative breast cancer patients. Systemic network analyses determined that the TGF- $\beta$  pathway is specifically active in CD44+ breast cancer cells and its inhibition induces their epithelial differentiation. CD24+ and CD44+ also demonstrated distinct responses to various therapeutic agents.

Our results demonstrate that the breast cancer cell phenotype is subject to regulation by genetic and epigenetic mechanisms and by the surrounding microenvironment. Thus, tumor progression is a dynamic and complex process that is influenced strongly by the intrinsic level of genetic instability in a given tumor at a given time and location. Understanding the molecular mechanisms responsible for breast tumor heterogeneity and specific targeting of each cell types within tumors will facilitate the development of more effective ways to treat and prevent breast cancer.

### **An integrative biology approach to predictive markers in breast cancer**

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Genome-wide analyses of the epigenome, genome and transcriptome in human breast cancers have revealed a wide range of molecular defects that likely contribute to cancer pathophysiology. Moreover, these analyses have revealed subsets of breast cancers carrying specific recurrent aberration signatures that progress and respond differently to specific therapies. Two of these subtypes – designated as basal and luminal/amplifier – often do not respond well to current chemotherapy and tend to recur or progress so that new treatment strategies are needed. We have developed molecular marker sets to define these two subpopulations so that patients with these tumor subtypes can be identified and offered access to experimental therapies. Hundreds of candidate therapeutic agents are under development in academia and industry that can be considered for subtype specific therapy. We have developed an in vitro system comprised of 50 well-characterized breast cancer cell lines that we are using to identify therapeutics that will be effective against the basal and luminal/amplifier breast cancer subtypes, develop assays that will predict individual responses within subtypes and identify mechanisms of response that may guide selection of complementary therapeutic agents. We have applied this approach to assess responses to ~25 approved or experimental anticancer drugs. Drug induced changes in viable cell number were assessed for each cell line at 9 different drug concentrations

and each analysis was performed in triplicates. Four general results emerge from these studies. (a) Many approved and experimental drugs show strong breast cancer subtype specificity. (b) Correlative analyses of associations between biological response and pretreatment molecular features yield multivariate predictors of individual response. The magnitude of biological response varies considerably between drugs and predictors of individual response are most accurate for drugs showing high variability of response between cell lines. (c) Drugs designed to target specific genomic defects are most effective in cell lines with those defects thereby validating the overall correlative approach. (d) Statistical analyses of markers associated with response identify the involved pathways and provide baseline information for pathway model development.

### Current challenges in biomarker discovery and validation

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In many ‘omics’ fields, extraordinary claims have been made about the accuracy of biomarkers for early detection of cancer, for predicting prognosis, and for predicting response to therapy. Yet such claims often turn out to be non-reproducible, and very few new markers have been brought out of the ‘omics’ pipeline into clinical application.

The disconnect between claims and reality can be explained in part by lack of attention to fundamental ‘threats to validity’ from ‘chance’ and ‘bias’. Those problems have been discussed before; the purpose of this presentation is to explain possible approaches to address them. While there is no ‘quick fix’ or simple solution – like ‘guidelines’ or ‘phases’ – progress could be made by attention to several critical topics:

First, every study, even ‘early’ ones, should be ‘reliable’ in the sense that a study should not contain fatal flaws due to chance or bias. Journals have a major role in assuring a study’s strength or reliability and its transparency.

Second, the quality of ‘specimens’ has an underappreciated role in helping to assure the reliability of a study’s results. The central concept is that, after specimens are collected, a ‘study’ *has been done*, regardless of whether the process was ever conceptualized

as a study. In other words, by the time specimens are collected, bias has – or has not – been hardwired into the study. Access to adequate specimens is the rate-limiting step in the field of biomarker research. High-quality specimens should be used even in ‘early’ studies, suggesting a compression of what we currently think of as ‘phases.’

Third, important ‘shortcuts’ may be available in marker research that are totally unavailable in drug development research.

Last, to solve the ‘culture clash’ that can occur when basic scientists and clinical researchers must collaborate in marker research, it may be useful to separate roles rather than to try to make each specialist into something he or she is not.

While molecular markers hold great promise for use in diagnosis, prognosis, and predicting response to therapy, that promise cannot be realized until we appreciate – and apply – appropriate “rules of evidence” to conduct and interpret research.

### **Can mouse models aid in the early detection of human cancer**

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There are several levels at which mouse models of human cancer might aid in development of assays for the detection of human cancer. In the context of genetically modified mice prone to develop specific cancer types such as ovarian cancer, evaluation of novel imaging strategies could be undertaken at various stages during the course of cancer progression. This would allow the sensitivity of the methodology to be evaluated. Similarly, body fluids can be collected at early to late time points during the course of cancer progression. Novel analytical technologies could be applied to such samples to inform as to sensitivity, specificity, and reliability. For example, if such an approach would have been utilized prior to the original evaluation of human blood samples from ovarian cancer patients by mass spectrometry issues of reliability might have been uncovered. For these possibilities, it is noteworthy that the cancers in mice would not even necessarily need to recapitulate the human cancers that were being modeled in any great degree. However, the greatest power

of such models would be in those that accurately mimic human cancers in histology, genetics and epigenetics. In such cases, discovery of new markers could be undertaken at the earliest stages of cancer in the mice in addition to their use to evaluate sensitivity, specificity, and reliability of an assay. One could also conceive of using a variety of mouse models of different cancer types to discover whether the marker(s) were specific for a given cancer type. One limitation of mouse models of human cancer is the lack of genetic diversity of the individual models since they are often in an inbred strain. The possibility exists that by cross breeding and creating a complex mix of genetic backgrounds in a mouse that still retained the cancer phenotype would be a means to model to some degree the diversity of the human population. This could provide for a large number of somewhat genetically diverse control samples (often lacking in human studies) for evaluation of test specificity.

### **Biophotonic risk stratification for colorectal cancer screening**

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Colonoscopy represents an important means for not just early diagnosis of colorectal cancer (CRC) but actually cancer prevention through identification and removal of the precursor lesion, the adenomatous polyp. However, less than 30% of colonoscopies are positive for adenomas meaning that more than 70% of these invasive procedures would be wasted. Therefore, risk stratification would be critical to develop a cost-effective screening technique. Many risk stratification techniques exploit field carcinogenesis, the proposition the genetic/environmental milieu that results in one area of the colon should be detectable, at least in some form throughout the colon. Previous studies have used morphological (adenomas, aberrant crypt foci), cellular (apoptosis, proliferation) or molecular (microarray/proteomics).

Our group has developed novel light scattering technologies called low-coherence enhanced light-scattering spectroscopy (LEBS) that allows us to detect the nanoscale structural abnormalities in histologically normal mucosa as a marker of field carcinogenesis.

Moreover, we have also developed a technology to selectively probe the peri-cryptal capillary plexus with 4 dimensional elastically scattered light fingerprinting (4D-ELF). We have published that both LEBS ultra-structural markers and the early increase in blood supply (EIBS) can be demonstrated in experimental models of colon carcinogenesis (azoxymethane-treated rat and MIN mouse).

We have performed rectal screening using both *ex vivo* and *in vivo* tests. From an *ex vivo* perspective, we took 2 biopsies from endoscopically normal rectal mucosa from 246 patients undergoing colonoscopy. The spectral markers were abnormal in patients harboring advanced adenomas ( $\geq 1$  cm) and intermediate (5–9 mm) but not diminutive adenomas ( $< 5$  mm). For advanced adenomas, our sensitivity and specificity 100% and 80%, respectively. The area under ROC curve (AUROC) was 0.895. In an independent convenience set of 40 patients total adenomas (there were no advanced lesions, and for this dataset we included diminutive lesions) had a comparable AUROC (0.707 versus 0.710, respectively). Current work is continuing on an *in vivo* has shown promise with performance better than *ex vivo* although the dataset is currently small.

From a technological perspective, an easier *in vivo* approach is to evaluate microvascular blood content (EIBS). We have previously shown that superficial (within 100  $\mu\text{m}$  of tissue surface) is a robust marker of field carcinogenesis in experimental models. We have manufactured a robust endoscopically compatible probe that can not only accurately measure pericryptal hemoglobin but determine hemoglobin oxygenation. We obtained 5 rectal readings from the endoscopically normal mucosa of 222 patients undergoing colonoscopy. We noted a 100% sensitivity and 75% specificity for advanced adenomas.

We believe that this approach will have relevance to other cancers that are characterized by field carcinogenesis. For instance, EDRN-supported studies on pancreatic and lung cancer have both been promising on initial clinical studies (300 and 120 patients respectively).

#### **Epigenetic biomarkers for detection of cancer: State of the science**

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Aberrant hypermethylation of the promoter region of genes is a frequent and early event in cancer cells. The hypermethylation is associated with loss of function of the gene. A number of genes, many implicated in important biological pathways, are known to be methylated in cancer. The average total number of genes methylated with functional significance in a tumor cell is unknown but might be reasonably estimated as several hundred. Global screens are leading to the elucidation of the cancer cell methylome. Mining of this data can improve current panels of genes used for early detection studies and extend such panels to provide signatures for differential diagnosis and prognosis. Sensitive methylation specific PCR technology exists that permits detection of gene methylation in tumor cells in tissue biopsies, urine, blood and other body fluids. Conceptually, tumor suppressor gene methylation is highly specific for neoplastic cells. Feasibility studies have demonstrated sensitive and specific detection of gene methylation in tissue biopsies and non-invasive body fluids from patients with cancer of an early stage when treatment can result in a better outcome. While, the rules of evidence for evaluation of detection and diagnosis are not yet as well-developed as for studies of therapy for cancer, certain challenges are apparent. For methylation-based detection, these include: the likely need for larger panels of methylated genes in detection, optimization and standardization of specimen processing and technology for analysis, further study of gene methylation in normal or non-neoplastic cells, knowledge of timing of methylation of a gene in regard to clinically significant disease, and the ability for differential diagnosis of the anatomical site of origin of a tumor in a body fluid. Strategies to meet these challenges are in progress. What is most important now is optimization and standardization of methylation-based detection and validation of clinical utility in larger, well-chosen populations. Studies within the EDRN include methylation-based detection of prostate and esophageal cancer in tissue, bladder and renal cancer in urine, colorectal cancer in stool, as well as lung and breast cancer in serum. Future areas of study will likely include: changes in the epigenome of the normal progenitor cells of cancer mediated by ageing and environmental influences, the earliest steps in the development of cancer, enrichment of rare tumor cells/DNA from body fluids, and the utility of additional types of epigenetic alterations in cancer cells as targets for the detection of cancer.