

Meeting Report

Aneuploidy Approaching a Perfect Score in Predicting and Preventing Cancer

Highlights from a Conference Held in Oakland in January 2004

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On January 23–26, 2004, a meeting, termed the *1st Conference on Aneuploidy and Cancer: Clinical and Experimental Aspects*, united about 70 cancer researchers at the Waterfront Plaza Hotel in Oakland. The conference was organized by two of us (Duesberg P, Rasnick D) to evaluate the theory that aneuploidy is sufficient to cause cancer. The abstracts or short papers of the participants are recorded in the *Scientific Program & Abstracts* booklet of the conference, which is available on request from the conference bureau at ssachs@uclink.berkeley.edu. An independent meeting report has since also been published in *The Scientist*.¹

THE ANEUPLOIDY-CANCER THEORY

The aneuploidy-cancer theory proposes that cancer is caused by the abnormal dosage of thousands of normal genes. This abnormal dosage of genes is generated by the gain or loss of specific chromosomes or segments of chromosomes,²⁻⁷ alias aneuploidy.⁸ According to this theory, carcinogenesis is initiated by a random aneuploidy which is induced either by carcinogens or arises spontaneously,^{9,10} but which is unlikely to generate the many new functions associated with cancer. However, the state of aneuploidy destabilizes chromosomes and genes because it unbalances highly conserved teams of proteins that segregate, synthesize and repair chromosomes. Thus the inherent instability of aneuploidy catalyzes a chain reaction of chromosome reassortments and rearrangements (Fig. 1).⁵ The theory predicts that chromosomal and genetic instability is proportional to the degree of aneuploidy and to the types of chromosomes that are unbalanced, which was confirmed at the conference by Fabarius et al. (Mannheim)¹⁰ and by Allison and Nestor (Toledo, OH).

Most of the random assortments of chromosomes generated by this autocatalytic karyotype variation will be either nonviable or less viable than normal cells, which have a chromosome assortment that has been refined and selected over 3 billion years of evolution. However, occasionally a random chromosome combination will be more viable in its habitat than a normal cell: This is the origin of carcinogenesis. These primary cancer cells will evolve further until they eventually progress to highly malignant variants by autocatalytic chromosome reassortments and selection for autonomous growth. As a result of this inevitably inefficient process, the somatic evolutions of cancer-specific aneuploidies will be slow—just like phylogenesis. Since the numbers and structures of chromosomes define a species, the aneuploidy theory holds that preneoplastic or neoplastic cells are new cell species. The aneuploidy theory also explains the idiosyncratic property of cancer cells and other aneuploid cells to overexpress or underexpress thousands of normal genes,^{11,12} because the normal dosage of thousands of genes is changed in each of these cell species by aneuploid chromosomes.^{5,7}

Further, the aneuploidy theory can explain, why most cancers and aneuploid cell lines eventually fall either into a relatively balanced and stable, near diploid ploidy-class or into a highly unbalanced and unstable, but maximally adaptable, near triploid ploidy-class⁷ (Fig. 1): Accordingly, a population of near diploid cancer cells maintains its identity by sacrificing much of the autonomy and variability of an ideal, maximally variable cancer cell for stability, which approaches that of diploid cells. By contrast, a near triploid population of cancer cells, theoretically the most unbalanced and variable cancer cells,³ maintains its identity and viability by maximizing adaptability but minimizing spontaneous variability.

To achieve this goal, the near-triploid, aneuploid cell must exclude or minimize chromosome combinations, which

- a. contain suicidal mutator genes,⁵
- b. unbalance the spindle apparatus and the normal numbers of centrosomes, as discussed at the conference by Fukasawa et al. (Cincinnati),¹³

- c. unbalance the biochemical assembly lines that synthesize nucleic acids and proteins,^{5,7} and
- d. corrupt “interchromosomal tethers,” an unexpected feature of karyotype stability introduced at the conference by Nagele and Kosciuk (Stratford, NJ).

These tethers were proposed to be responsible for the “surprisingly stable propagation of abnormal karyotypes (in view of a–c) during proliferation of immortalized cancer cells.”

Aneuploidy also catalyzes gene mutations by corrupting protein teams that synthesize and repair DNA and synthesize nucleotide pools.^{68,69} Accordingly, the many gene mutations of cancers are inevitable consequences of aneuploidy. Most of these would be irrelevant to carcinogenesis,⁷⁰ while some might confer selective advantages to a given cancer cell-species—just like some of the 1.42 million mutations that set apart any two humans may confer advantages to a given individual of the human species.⁷¹

By contrast, the competing mutation theory sees cancer cells as mutants of their progenitor cells. But, unlike all conventional mutations or mutant organisms cancer cells are notoriously unstable.^{5,7,14} They progress spontaneously from very low degrees of malignancy to very high degrees, or change spontaneously from drug-sensitive to drug-resistant, or metastasize from their native location to another. Moreover, despite efforts of over 20 years, it has not been possible to transform any diploid animal or human cell into a cancer cell with any combination of mutant genes from cancers tested so far.^{4,5,15-17} Failures to find overexpression or even any expression of the hypothetically “dominant oncogenes”¹⁸⁻²⁰ in cancer cells further undermine the view that these genes can cause cancer (see also below).^{3,6,12,21,22}

Likewise one or several hypothetical cancer genes have been artificially introduced into the germ line of transgenic mice, but the individual cancer-risks of these mice are only marginally higher or even the same as that of controls, and are both age- and strain-dependent, and the cancers of these mice are clonal, indicating that these genes are not sufficient for carcinogenesis.^{5,7,15,23-27} A calculation of the cellular cancer risk of these mice makes this point even more obvious. Since cancers originate from single cells,^{11,28} and since mice consist of about 5×10^{10} cells and have renewed many of their cells by the time they may develop cancer, their cellular cancer risk is less than 5×10^{-10} . This extremely low cellular cancer risk further diminishes the argument for a direct role of such genes in carcinogenesis. Thus the mutation theory is burdened with numerous paradoxes—the hallmark of a flawed theory.

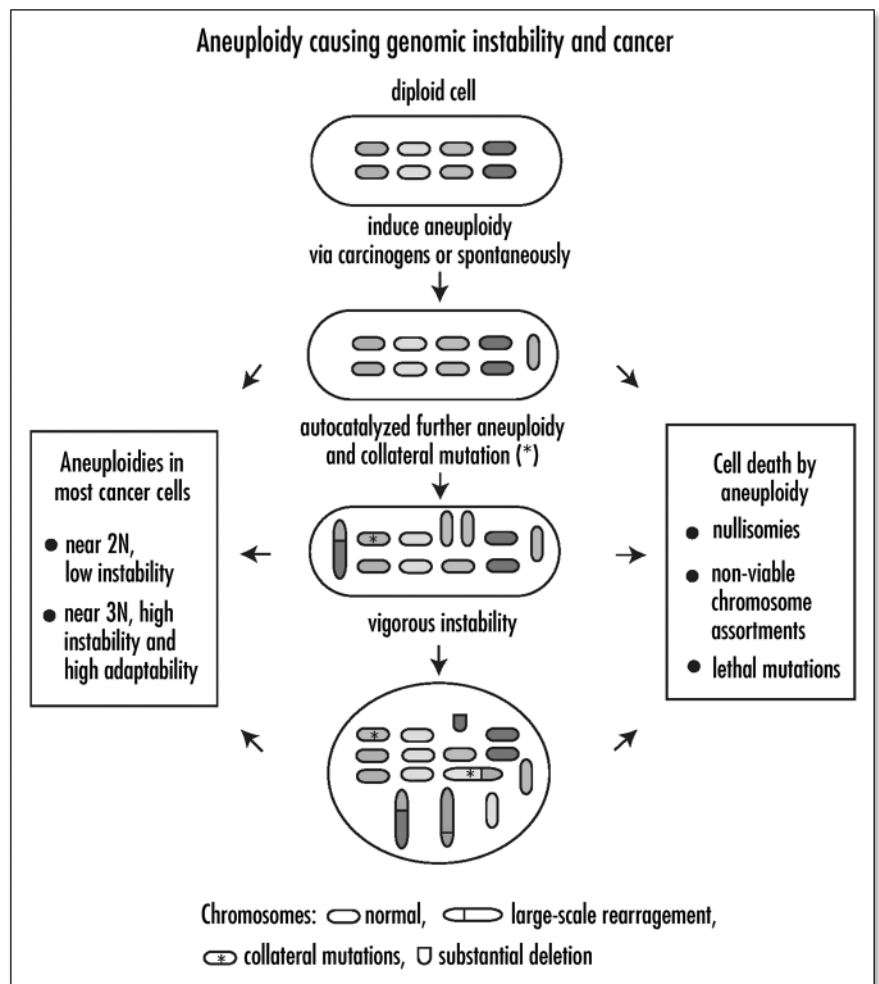


Figure 1. Carcinogenesis and genomic instability by aneuploidization. According to the aneuploidy-cancer theory carcinogenesis is initiated by a random, spontaneous or carcinogen-induced aneuploidy. The state of aneuploidy unbalances highly conserved teams of proteins that segregate, synthesize and repair chromosomes. The resulting chromosomal and genetic instability causes a chain reaction of autocatalytic chromosome reassortments and rearrangements. Most of such aneuploid cells will die, because most cells with random chromosome assortments will be nonviable or less viable than diploid cells with a 3 billion-year history of karyotype evolution. However, occasionally a chromosome combination will emerge, which has a growth advantage over its diploid progenitors—the origin of a cancer cell. According to this theory aneuploid fitness will eventually divide most aneuploid cancer cells into two classes: a near-diploid, relatively balanced class approaching the stability of diploid cells, and a near-triploid, maximally unbalanced and unstable class, which derives fitness from maximal adaptability.

ANEUPLOIDY VERSUS GENE MUTATION THEORY AT THE OAKLAND CONFERENCE

Despite numerous collisions between the two theories at the conference, the aneuploidy theory held its own on several grounds.

Diploid Cancer? The finding of a diploid solid cancer would support the mutation theory, which holds that cancer is a consequence of mutation.^{4,11,29-31} However, no such cancers were described at the conference, except for suggestions from the floor that colon cancer with microsatellite instability may be diploid. But, even this example was quickly eliminated by Waldman et al. (San Francisco) with a gene array-based variation of comparative genomic hybridization. This method detects abnormal dosages of thousands of genes, termed “frequencies of DNA copy number alterations.”³² With this technique Waldman et al. found aneuploidy in both early “microsatellite stable and unstable tumors ... (20% versus 5%, respectively),”

and concluded that “chromosomal instability is an early event in colorectal carcinogenesis.”³²

Is the Aneuploid Dosage of Thousands of Unmutated Genes or the Mutation of a Few Specific Genes Causing Cancer? According to the aneuploidy theory, the many phenotypes of cancer cells are generated by the over- or underexpression of thousands of normal genes, which depends on their aneuploid dosage. By contrast, the mutation theory predicts that one or a few mutant genes are the cause. Using microarrays with thousands of gene probes, Miklos and Maleszka (Sydney) and Szallasi et al. (Boston) measured (a) the aneuploid dosage and (b) the mRNA expression levels of thousands of genes in various cancers. Analyzing genomic imbalances of near-diploid and highly aneuploid multiple myelomas Miklos and Maleszka concluded, “residents of amplicons and deletions have their RNA outputs determined by their non-diploid status.” Szallasi et al. found in “immortalized, non-malignant” breast epithelium “a significant number of differentially expressed genes relative to normal cells” and “a new burst in gene expression” during “malignant transformation.” Ried et al. (Bethesda) also observed non-correlations between the dosage and expression of certain genes in colon cancer, signaling aneuploidy-mediated negative and positive gene regulation (see also ref. 33).

In view of the multiplicity of the abnormally expressed genes and the inherent instability of aneuploidy, Miklos, a veteran aneuploidologist,³⁴ and Maleszka questioned the merits of targeting hypothetical “oncogenes and tumor suppressor genes” for cancer therapy: “It is rarely the single gene that is the culprit, it’s the imbalanced genome that’s the problem. ...Three decades of cancer research reinforce one conclusion; the prioritized single gene approach is assuredly almost always doomed, both diagnostically and therapeutically.” Likewise Cooper (Ann Arbor) presented experimental evidence for a “continuum model” of the cell cycle, which holds that the cycle is regulated pluralistically by numerous cooperative enzymatic reactions, rather than by singular rate-limiting reactions. For example, he showed that human and murine leukemic cells go through G₁ phase without the hypothetically rate-limiting dephosphorylation and subsequent re-phosphorylation of the retinoblastoma protein.³⁵ Thus, his model also called into question the “single gene approach” for cancer treatments.

Aneuploid Chromosomes and Centrosomes: Which One is First? The findings of up to 8 centrosomes in various human cancers, instead of the normal 2, by Brinkley et al. (Houston), Doxsey (Worcester), Fukasawa et al. and Lingle et al. (Rochester) were also interpreted as a challenge to the aneuploidy theory. As-yet-undefined mutations were proposed to increase the numbers or alter the structures of centrosomes, which would then cause aneuploidy by asymmetric mitoses. However, the alternative explanation that aneuploidy would generate abnormal numbers and structures of centrosomes was not ruled out.³⁶ According to Doxsey this must be a difficult call—time-wise at least: “We think that as soon as you have an extra copy of a centrosome, in the next [cell] division you’re going to have aneuploidy.”¹

Is Chromosomal Instability Caused by Aneuploidization or by Mutation? The aneuploidy theory predicts that a random, carcinogen-induced or spontaneous aneuploidy initiates carcinogenesis via chromosomal destabilization (Fig. 1). This prediction was challenged by Steinbeck (Kiel) with evidence for “pathological telophases” preceding aneuploidy, and by Murnane et al. (San Francisco) and Stampfer et al. (Berkeley) with evidence that defective telomeres generate aneuploidy. According to the report on the conference from *The Scientist*, Lengauer et al. from the Johns Hopkins University

(who unfortunately had to cancel an invitation to the conference) also challenged the aneuploidy theory with a primary mutation of cyclin E, which would cause “chromosomal instability” in the case of colon cancer.^{1,37} However, Lengauer acknowledged, “The mechanism by which higher cyclin E levels create chromosomal instability remains ‘unsolved.’”¹ Indeed Lengauer et al.’s data support the aneuploidy theory even better than the mutation theory, because the new cyclin mutation was found in only 12% (22/190) of the colon cancers with “chromosomal instability” and in only 8% (4/58) of adenomas,³⁷ which are already chromosomally unstable according to the same group.³⁸ Thus aneuploidy or chromosomal instability seemed to have been present in 100%, but mutant cyclin only in 8–12% of adenomas and colon cancers, respectively.

Wright et al. (Dundee) provided further support for mutation-independent “chromosomal instability” from an unexpected angle. Using various forms of ionizing radiations to generate chromosomal instability, including X rays and alpha particles, Wright et al. demonstrated that the target, chromosomal instability alias aneuploidy, is >1000-fold bigger than the target, gene.³⁹ Thus radiation induces chromosomal instability via aneuploidy >1000-times more effectively than via gene mutation—either by deleting or rearranging chromosomes or by damaging components of the spindle apparatus such as centrosomes.⁷

Drug-Resistance of Cancer Cells via Mutation or Specific Aneuploidies? The idiosyncratic property of cancer cells, to become resistant to chemotherapy, is the nemesis of cancer chemotherapy.⁴⁰ This phenomenon is generally blamed on gene mutations that generate or activate drug-resistance genes.⁴¹ But the paradox that cancer cells become drug-resistant within weeks to months, whereas comparable populations of normal diploid cells of the same patient or of normal human cells in culture remain sensitive, is as old as chemotherapy.^{40,42} In view of this it has recently been proposed that cancer cells survive chemotherapy because their genomic instability generates mutations in apoptosis genes.⁴³ However, the necessary mutator genes have only been found in a small minority of cancer cells.^{5,7}

Now, the aneuploidy theory offers a new solution to this old paradox. Considering that aneuploidy is ubiquitous in cancer and inherently unstable, drug-resistance could be generated by specific chromosome reassortments rather than by gene mutation.^{44,45} Indeed, experiments comparing highly aneuploid Chinese hamster and mouse cells to their diploid progenitors have already shown that only aneuploid cells become drug-resistant at detectable rates.^{44,45} But, the question whether specific aneuploidies are involved could not be answered so far because of the high chromosomal instability of aneuploid Chinese hamster cells, as described by Fabarius et al. at the conference.⁴⁶

In view of the unexpected observation, that the karyotypes even of highly aneuploid human cancer cells are about 1–2 orders more stable than those of highly aneuploid Chinese hamster and mouse cells, two of us (Li R, Duesberg P) have now investigated human cancer cells for drug resistance-specific aneuploidies. At this point 5 out of 5 clones of puromycin-resistant human colon and breast cancer cells were each found to contain 2 or more specific aneuploidies that were not found in clones of untreated progenitor cells. In view of this, we conclude that the high rates of “mutation” of cancer cells to drug resistance are due to specific chromosome assortments generating drug-resistant phenotypes.

CLINICAL RELEVANCE OF ANEUPLOIDY TO CANCER

If cancer were caused by aneuploidy, malignancy should, in a first approximation, be proportional to the degree of aneuploidy (Fig. 1), and, in a second approximation, to cancer-specific aneusomies.¹⁰ Moreover, aneuploidy should precede malignancy (Fig. 1) and thus cancer should be preventable by detecting and eliminating precancerous aneusomies. All three of these predictions were generally confirmed at this conference.

Malignancy Proportional to Degrees of Aneuploidy. Several conferees including Auer et al. (Stockholm), Boecking et al. (Duesseldorf), Eastmond et al. (Irvine), Giaretti et al. (Genoa), Haas (Vienna), Knoesel et al. (Berlin), Petersen et al. (Berlin), Rabinovitch (Seattle), Reith and Sudbo (Oslo), Ried et al. and Waldman et al. showed data demonstrating more malignancy with more aneuploidy. In his opening lecture and in related articles Auer pointed out “Aneuploid cancers are generally considered to be more dangerous and aggressive than their diploid (meaning near-diploid, see above) counterparts.”⁴⁷ Studying aneuploidy in large numbers of cervical and colon cancers in all stages of carcinogenesis Ried et al. found that, “Chromosomal aberrations are key events in the initiation and progression of cancer” and that, “The average number of chromosomal copy number alterations increased with increasing stages of cellular dysplasia (see also ref. 48).”

However, there are exceptions, as for example some leukemias described by Haas, in which specific aneusomies of near-diploid leukemias were treatment-wise more “unfavorable” than gross aneusomies of some highly aneuploid counterparts. Despite these exceptions the principle, that the degree of aneuploidy is proportional to that of malignancy, also applies to leukemias as for example to lymphocytic leukemias⁴⁹ and acute myeloid leukemias, in which “complex karyotypes predict an extremely poor prognosis.”⁵⁰

Cancer-Specific Aneusomies. Several participants also detected cancer-specific aneusomies and chromosome rearrangements using either comparative genomic hybridization or fluorescent in situ hybridization of interphase nuclei with chromosome-specific DNA probes. For example Peterson et al. described specific aneusomies in lung cancer, and Knoesel and Peterson et al. as well as Giaretti et al. in colon cancer. Ried et al. provided the most detailed evidence for “strictly conserved distribution of genomic imbalances,” alias aneusomies, in cervical and colorectal cancers.

In addition Chin and Gray et al. (San Francisco), Knoesel and Peterson et al., Rabinovitch, Ried et al. and Waldman et al. were each able to correlate specific aneusomies with specific stages of breast, colon, esophagus and cervical cancer. The early human aneusomies were simple and the late aneusomies were more complex—just as was reported at the conference by Fabarius et al. in experimental carcinogenesis of Chinese hamsters initiated with nitrosourea.¹⁰

Cancer Therapy and Prevention Based on Precancerous Aneuploidy. According to the aneuploidy theory, the slow and inefficient evolution of a random preneoplastic aneuploidy into cancer-specific aneusomies is the Achilles heel of carcinogenesis (Fig. 1). The unexpected success of several clinical cancer researchers to distinguish—solely on the basis of aneuploidy—prospectively dangerous, precancerous neoplasias from “atypical” or indeterminate, but benign neo-, hyper-, meta-, and dysplasias turned out to be the absolute highlight of the conference.

In his opening lecture Auer was the first to point out that “Swedish clinicians already consider aneuploidy in determining

treatments for various tumors.”¹¹ A collaboration of Auer’s team with Ried made the case for the early detection of breast carcinogenesis based on the “DNA content” or the “DNA index,” alias the degrees of aneuploidy. For example, they showed that adenomas with “histogram type I,” i.e. near-diploid or diploid, “have an excellent prognosis with 95% probability of 10-year survival. In contrast patients with histogram type IV,” i.e., highly aneuploid, “have an extremely bad prognosis with only 31% of 10-year survival (see also ref. 51).” But, if analyzed on the basis of the competing oncogene-mutation theory, only 44% of the breast adenomas with “DNA histogram IV,” “showed an amplification of one or more oncogenes studied (*c-erb 2*, *cyc D1*, *int-2*, *c-myc*, *MDM 2*).”

Auer and Ried et al. also pointed out that there was “no clear correlation between DNA histogram type and axillary node status,” alias metastasis. This is consistent with the independent progression of cancer-specific phenotypes as originally described by Foulds,⁵² and likewise with independent chromosome assortments generating heterogeneous phenotypes in clonal populations of aneuploid cells (Fig. 1).

Boecking et al. carried out a prospective study to determine the progression to malignancy of Papanicolaou (cervical) smears with “atypical squamous cells of unknown significance (ASCUS),” using “DNA-image cytology” for the detection of aneuploidy. The technique, which measures cellular DNA content, is in principle the same as Auer’s. The median interval between the initial “atypical” smear and the subsequent histological grading into “cervical intraepithelial neoplasia (CIN) status I-III” was 3 months.⁵³ During this interval 65% of the aneuploid atypical samples, but only 35% of the total atypical samples developed a neoplastic status of > CIN II. Moreover, “DNA image cytology” correctly predicted that the majority of diploid or near diploid atypical samples did not progress to a neoplastic state, “non-progression within 6 months was 85%”. In a parallel, prospective study Boecking et al. also investigated the cancer-risk of “suspicious oral lesions,” such as leukoplakias, based on aneuploidy. In that study they found the “positive [cancer] predictive value” of aneuploidy to be “100%” and the “negative [predictive value to be] 99%”—compared to a “positive predictive value of 98% and negative predictive value of 98.5%” by conventional cytology.^{54,55} Based on “the high prognostic validity of DNA-aneuploidy” Boecking et al. concluded, “aneuploid lesions should immediately be controlled histologically or removed”.

By examining “normal, dysplastic and cancerous [cervical] Pap smears” for aneuploidy with fluorescent probes for chromosomes 3 and 17, Eastmond et al. observed “a sequential pattern”, in which “significant increases in both tetraploid and aneuploid cells were seen with disease progression. The proportion of women exhibiting elevated frequencies of tetraploidy and aneuploidy increased from 1/26 and 0/26 among women with normal Pap smears to 20/39 and 22/39 for women with high-grade cervical lesions.”

Reith and Sudbo investigated retrospectively the risk of throat cancer based on the presence of aneuploidy in premalignant “oral leukoplakia.” The degree of aneuploidy was deduced from the “DNA content” of premalignant cells, which was measured photometrically, essentially as described by Auer and Boecking. Reith and Sudbo found that “23 of 27 (84%) aneuploid cases” developed oral squamous cell carcinoma within 8 years after diagnosis. By contrast, “only 3 of 103 (3%) of diploid cases” developed carcinomas during the same period of observation (see also refs. 56 and 57). The authors concluded, “these studies place ... aneuploidy as the cause of malignant transformation at the beginning of this process.”

According to a commentary on their work by Greenspan and Jordan, “The only other predictor of squamous-cell carcinoma, according to multivariate analysis, was tobacco use,”⁵⁸ which is known to be aneuploidogenic.^{59,60} Zhang and Smith (Berkeley) made an independent case in point for the induction of aneuploid leukemias by occupational exposure to benzene.

“To examine the predictive value of aneuploidy” and of tetraploidy in Barrett’s esophagus, Rabinovitch followed a cohort of 322 patients for up to 15 years. Ploidy was measured by flow cytometry. Rabinovitch found, “Among patients with negative, indefinite or low grade dysplasia, those with neither aneuploidy nor tetraploidy had a 0% 5-yr cumulative cancer incidence, compared with 28% for those with either of these findings.”⁶¹ A similar result was obtained in predicting cancer in ulcerative colitis patients based on preneoplastic aneuploidy. In both diseases, “chromosomal instability,” measured with fluorescent probes for centromeres and for selected chromosome segments, preceded changes in DNA ploidy detectable by flow cytometry. On this basis Rabinovitch concluded, “Neoplastic progression in ulcerative colitis and Barrett’s esophagus is facilitated by an underlying process of chromosomal and genetic instability that culminates overtly as aneuploidy;” and proposed, “Knowledge of these intermediate stages in neoplastic progression may help manage patients with ulcerative colitis and Barrett’s esophagus to more effectively prevent cancer.”

CONCLUSIONS AND CONCLUSIVE OPTIMISM

The unexpected coincidence that several, independent labs demonstrated the clinical relevance of aneuploidy, was the absolute highlight of the conference. Indeed, the clinical relevance of aneuploidy was perceived by most at this conference as a liberation of the aneuploidy theory from the competing gene mutation theory. For decades, aneuploidy theory played the subordinate role of advancing hypothetical, poorly defined oncogenes via chromosome gains, and eliminating hypothetical tumor-suppressor genes via chromosome losses.^{31,62-65} Now it had advanced from this bystander role to an independent cause of cancer. At last, cancer research more generally may have been liberated from the decades-long grip of the gene mutation theory.

In addition to the already proven, clinical gains, further improvements in predicting the cancer-risk of indeterminate hyper-, dys-, meta- and neoplasias can now be expected from applications of the aneuploidy theory. For example, near-diploid, aneuploid cells that cannot be distinguished from normal diploid cells by DNA-content methods could be identified with fluorescent chromosome-specific probes. Further, among equally aneuploid precancerous cases, low-risk, stable aneuploidies could be distinguished from high-risk unstable aneuploidies by measuring the chromosomal scatter-indices of aneuploid cell populations.⁶⁶ This chromosomal “stemline scatter index” is exactly what Auer and Ried et al. have already used to distinguish “low and high malignant subtypes” of “diploid and tetraploid” breast adenocarcinomas (see also ref. 47). Indeed, Boecking and Chatelain have written—back in 1989 (!)—“the range of chromosomal abnormalities, not the modal chromosomal aberration, correlates with the malignant potential of a [cervical] tumor.”⁶⁷ Considering that many clinical cancers fall into the near-diploid class (Fig. 1), which is undetectable by DNA-content methods alone, but is now detectable by chromosome-specific probes—and that aneuploidies, which are detected by DNA-content methods, can now be sorted into relatively stable and highly unstable subclasses based on their chromosomal

scatter indices, we might soon close the remaining gaps in the detection of precancerous lesions by the various, already established DNA content-methods.

Although the era of clinically predictive aneuploidy has just begun, it seems possible now that a danger-index of aneuploidies can be developed, which identifies and ranks precancerous aneuploid chromosome combinations and rearrangements according to their abilities to generate chromosomal instability, autonomous growth, and drug-resistance.

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