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Genomic Instability in Cancer II: 4N-Skewed (90˚) Reductive Division via Fragile Sites to Fitness Increase for Solid and Hematological Cancer Beginnings

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Abstract

The objective herein was to connect the ontogeny process of diplochromosomal, amitotic, 4n-skewed division-system, to cytogenetic deficiency lesions in satellite, repetitive DNAs, especially in the chromosomal fragile sites, some 100 distributed over the genome. These latter studies had shown that chemical induced replication-stress led to un-replicated lesions in these fragile sites, which from inaccurate repair processes caused genomic instability. In the chain of events of the ontogeny process to the special tetraploidy, it was proposed that primary damaged human cells could undergo replication stress from repair-process present during cell replication, a suggestion verified by X-ray damaged cells producing the unstable fragile sites (see text). The cancer-importance for therapy is recognition of cell cycle change for the 4n derivative fitness-gained, diploid progeny cells. An open question is whether RB controlling G1 to S-period is mutated at this suggested tumorigenesis initiating phase, and if so, with what consequences for therapy. The fragile site studies further showed that repair of repetitive DNAs could produce two types of genomic changes: single gene mutations and CNVs, which were here shown to be chromosomally located on "borders" to repairing satellite lesions. This genomic placement was found to correspond to mutations identified in tumor sequencing (p53, Rb, MYC), favoring a bad luck location for their cancer "mutational nature". The CNVs in cancers, are here seen as molecular expressions of long-known cytogenetic HSRs and DMs also with demonstrated origin from amplifications of single genes. Over-expression of oncogenes was hinted of being from duplications, but *Drosophila* genetics demonstrated the opposite, *gene inactivation.* The reduced eye-size from dominant, BAR-Ultra-Bar-eye phenotypes, was caused by duplications, inactivating the

genetic system for eye-size. The finding of CNVs showing "evasion" of the immune system suggests, *inactivation of immune-determining genetics*. Since mutated genes on borders to satellite DNAs are a fact in hematological cancers, the 4n-skewed division-system is suggested to replace debated leukemogenesis with fitness-gain from molecular mutations. For these cancers the question is how normal bone marrow cells attain genomic damage for special tetraploidy, which was referred to studies of cells moving in artificial marrow-like substrate, needing serious attention.

Keywords

Centrifugal 90˚ Turn, Centrosome Absence, Mitotic Slippage Process, Diplochromosomes, Mutator Mechanism, Satellite DNA Mutations, Fragile Site Instability, Repair Mutations, Copy Number Variants, Chromosome Nuclear Domains, Hematologic Translocations, Density Bone Marrow Substrate, Abnormal Laminar Proteins, Chromosome/Gene UPD, Haplo-Insufficiency

1. Introduction

Dogma: *first is the supposition*, *then the assertion*, *then the faith*, *and finally the fanaticism* (Virchow ~1858)

In a recent publication [1] we discussed the data that led to the realization of a special type of tetraploidy (4n-skewed-division-system) that could give rise to fitness-gain in normal human cells, a first required hallmark of tumorigenesis [2]. Normal proliferating cells are under strict controls against genomic changes inclusive of tetraploid divisions [3], but a special 4n type with 46 diplochromosomal, 4-chromatid chromosomes, unexpectedly showed divisions in normal human cell populations [4] [5]. This surprise could be chromosomally followed, and was identified to be a two-step genome reductive sequence to karyotype, normal diploid cells [6] [7]. We note that an "evolutionist", describing meiotic alternatives for genome reductive divisions, correcting accidental genomic doubling [8], invoked diplochromosomal structure by: "each chromosome is represented by four copies-", in essence meaning 4-chromatid chromosomes.

This meiotic alternative from evaluation of primitive unicellular organisms [9] supports our conclusion of a non-meiotic, but an amitotic, evolutionary conserved archaic derived, 2-step reductive division-system from diplochromosomal cells. Further evolutionary evidence comes from hermaphroditic species, which also show the first division to be equational and, the second division to be reductive [10]. Further observations *in vitro* of lightly seeded cultures with normal human cells, made to carry DNA-damage, showed that rarer, 4n, diplo-chromosomal nuclei were in a 90˚ changed position relative to the cell axis, the cytoskeleton, a phenomenon for certain extraordinaire. This self-inflicted 4n, nuclear rotation preceded the 2-step, genome reductive division sequence, and gave rise to progeny cells in a perpendicular orientation relative to neighboring

cells [11] [12]. These karyotype normal diploid cells, demonstrated long-lasting gain in fitness, out-growing the normal cells, but note, they were not immortal, they had not gained activation of the telomerase gene [13]. They could however, undergo oncogenic transformation, experimentally shown by a telomere "crisis", surviving clone, responding to oncogene RAS-exposure with tumors in nude mice [14]. But before going into one possible explanation, for the self-inflicted 4n, nuclear 90˚ turn, tetraploidy in general suggested for tumorigenesis, has to be considered. To avoid repeat of already published data and references from the 4n-skewed division-system [1], we have summarized the special-4n ontogeny process and its consequences from the skewed 90˚ genome reductive system in Table 1 & Table 2.

Table 1. Ontogeny of the 4n-skewed, amitotic division-system.

A	This genome reductive system has only hitherto been observed in normal human cell populations, carrying DNA-damage
B	DNA-damage repair caused nucleotide lesions in Fragile Sites (ref). Thus, a cell in our cell population, but with special type DNA-damage, capable of starting repair during S-period.
C	This repair slows-down progression of S-period, resulting in un-replicated Late DNA, the satellite DNAs, now variously affected by deletion-type "mutations"
D	These mutations in their own right, will trigger repair-processes, and the question of stable or unstable solutions, is a real issue for genome/chromosomal instability (GCIN), "demanded" for progression
E	This aberrant S-period can also affect G2 phase time-wise with consequence deteriorated mitosis program, Cyclin B and CDK-1 with chromatin structured diploid cells directly in G1-phase
F	G1-phase becomes time-reduced, because of no de-condensation of chromosome structure with diploid cells entering a second S-phase, seemingly not interfered by checkpoint controls
G	This S-period completed with genome-wide down-load of extra cohesin, forming 4-chromatid diplochromosomes, the chromosomal structural base for the 4n-skewed division-system
H	Division-consequence: stable/unstable repair of satellite DNA deletion-mutations in diploid offspring cells, a potential mechanism for genome instability-associated genetic/epigenetic variability for tumorigenesis.

Table 2. Self-inflicted 90˚ re-orientation of the 4n/8c nucleus before division: consequences.

A	Inheritance, in resulting, clonal, karyotype normal fitness-gained diploid cell propagation
B	4n nucleus in perpendicular position relative to cell polarity axis, the cytoskeleton
C	4n-mother cell becomes physically withdrawn from cell-to-cell adhesion-contacts by division in a 90˚ turn relative to the cytoskeleton
D	Destruction with loss/displacement of cell adhesion proteins, E-cadherin and beta-catenin
E	Cell polarity change/loss is a cancer-associated evolutionary developing, permanent feature
F	Whole complement segregation followed by simple fission division places fitness-gained cells in a 90˚ orientation relative to surrounding tissue cells
G	Cell-freedom from contact inhibition and gain of motility
H	Original cytoskeleton in 4n-mother-cell "crushed" (destroyed) in 4n cell "apical-basal" division-system, demanding rebuilding in offspring cells
I	Cytoskeleton rebuilt in a perpendicular orientation relative to original cell axial orientation
J	Restructured cytoskeleton confers cell-polarity and cell-shape (morphology) change on clonally propagating fitness-gained cells
K	Cell axial change in "epithelial-mesenchymal-transition (EMT)" is from 90˚ 4n-skewed division-system

Comment: the present lack of a distinction between poison-tetraploidy and 4n-skewed division system, is causing confusion in what is cancer-real and what is not, and should be recognized.

2. Tetraploidy in Tumorigenesis

In general, this subject matter is either discussed from detailed specific cellular events (*i.e.*, multipolar spindles) or is all inclusive of such happenings, the broad hand approach, rather well presented in a last year article [15]. Few if any focuses with question on how and why the doubling of the genome can occur in note, normal human cells where cancer starts, and be a tolerated genomic change [16]. But note, these experimentally endoreplicated tetraploid cells do not divide, and have originated the "tetraploid paradox": proliferation in cancer cells but not in normal cells [17] [18]. Spindle poison induced 4n cells in diploid cancer cell lines undergoing asymmetric divisions, are herein not accepted as model for cancer initiation in normal human cells [19]. This exclusion also applies to spindle poison used on normal cells, which produce diplochromosomal cells [17], because such treatment results in accumulation of normal cells arrested in metaphase (used in karyotype analyses), and from there the genome becomes doubled by endoreplication to 4n cells. This start from bichromatid diploid cells (2n/4C), avoids/evades the occurrence of molecular mutations (for fitness-gain), characterizing a start (initiation) from genomic damaged, replicating normal cells (2n/2C) to the 4n-skewed, unique division-system. The fact that *in vivo* pre-cancers are initiated from clonal cell proliferation, is supporting evidence for the latter division-system, because it indicates selection of molecular mutations giving rise to fitness-gain (see below). The initial absence of chromosomal aberrations (normal karyotypes) in the fitness-gained cell population, is also commensurate with the limited tolerance of genomic change in normal human cells [16]. To these considerations it is emphasized that the self-inflicted 90˚ nuclear reorientation in the 4n-skewed system, has published, photographic illustrations, and that the subsequent amitotic divisions achieve escape from spindle checkpoint controls [12] [13] [14]. These features are beneficial to establishment of "new cell growth", but not yet recognized for their potentials for cancer initiation and evolution [15]. Lastly, the measured 90˚ turn of hyperplastic cell growth (fitness-gained cells) relative to the basal cell membrane [20], is a verification of the 4n-skewed division system in the initiation of this pre-cancerous cell proliferation, conserved in the human genome from perpendicularity in divisions of unicellular organisms [1] [10]. These crucial differences between the two types of tetraploidy, is presently not acknowledged, and is causing bad confusions in the concept of initiation in tumorigenesis.

3. How Is the Self-Inflicted Nuclear 90˚ Turn Accomplished?

When everything else fail, look to yeast (unknown).

Two species of yeast, *Saccharomyces cerevisiae* and *Saccharomyces pombe*, have historically been in the center of investigations into "cell division irregularity" responses from their respective, diploid *budding* and haploid *fission*, mechanisms of divisions. The latter fission-division was a popular subject in the explanation of vestiges in different animal phyla of the evolutionary tree. But these

"wise" men, did not report on a most peculiar nuclear, oscillatory movement expressed in fission division [21]. This latter author reported that upon fusion of two haploid cells each with 3 chromosomes, there was an immediate, "diploid" nuclear spinning-response. This was achieved by a spindle pole body (centrosome-like) at one nuclear pole, being attached to the cell wall by dynein and microfibers, giving the diploid nucleus freedom for a series of fast turnarounds. At the end of the spinning, two smaller, haploid nuclei were displaced in a perpendicular placement to the pre-spinning cell axial orientation (90˚ changed position). The authors further concluded that there was absence of a synaptonemal complex, but that 24 "linear elements" were present during this primitive, *genome reductive division system* of diploidy to haploidy.

This detailed nuclear spinning-scenario, immediately brought to mind, "artefactual" observations of cells spinning in lightly seeded cultures, where the 4n-skewed division-system was being observed [22] [23] [24]. Two "for sure" spinning cells had amusingly been observed in cultures still incubator-warm. One axial cell-end was apparently attached to the cultural surface. These events were seen as artifacts, but were immediately re-called upon the discovery of the *S. pombe* report. Specifically, the self-inflicted 90˚ nuclear re-orientation, now appeared to have an explanation. The fast spinning was interpreted to produce *centrifugal* force, which could tip the axial oriented nucleus to a "parallel" placement relative to the cytoskeleton axial orientation. If true, the spinning mechanism would be a genome conserved trait in the human genome. But it still is unknown of what triggers the 4n-cell to perform this very likely pre-mitotic-meiotic behavior, suggested to correct "archaea" accidental genomic doubling [25]. Moreover, the nuclear internal cell division, occurred "independent of spindle microtubules". These authors concluded that the nuclear fission, division-system might "reflect an evolutionary intermediate state between bacterial and arcane chromosome segregation", before evolution to spindle apparatus, dependent mitotic division.

4. Observations of Diplochromosomal Tetraploidy

Our first observation of 4-chromatid chromosomes was in 1965 [26], which in subsequent years was sought from diverse treatments, without success, and then they were observed as spontaneous events in near-senescence, decades later [4] [6] [7]. At that senescence associated late population doubling time, the telomeres from normal attrition had reached repair instability, which suggested the observed tetraploidization [27] [28]. From these observations it was assumed that DNA damage in young cells would lead to the special 4n cell-phenotype, which was explored for cells, having been exposed to nutrition deficit, cultural medium, followed by recuperation in complete medium [1]. And indeed diplochromosomal cells were present in such normal cell populations [28], and as expected there was also presence of foci with fast growing diploid cells with normal karyotypes. These cells also showed cell polarity change, which a century ago, was concluded to be an "inborn" characteristic of the cancerous process

[29], and others, expressed it to be the "gateway" to tumorigenesis [30] [31] [32]. Note, our observations were from carcinogen-free experiments. Interestingly, earlier observations of diplochromosomal cells, were consequences from normal human cells, having been exposed to seven different carcinogens [33]. Thus, it now is likely that carcinogens can be cancer inducing via an initiating 4n-skewed division-system.

4.1. Aneuploidy versus Mutation Theory (MT) in Tumorigenesis

Science principles are based on factual-data, which experimentally however, are never absolute, but within the goal of evidence-based facts with probability values as close as possible to 1.0. We have drawn attention to the fact that suppositions can over time change to "reality" in our minds, which is a form of dogma development [1] [34]. We discussed a persistent dogma in our concepts of how frequently mutated genes (mountain mutations) in tumor sequencing are formed, and our accepted function of driving cancer evolution [1]. There is not a "stich" of evidence for this belief, on the contrary, the original articles carefully, suggested these characteristics, of being interpretations, and specifically used the word, an *idea* [35] [36]. Furthermore, the same laboratory, was later seeking evidence for the idea, by showing that these mutations would create fitness-gain for the tumor driver capacity. This failed idea [37], causing lack of normal cell culture demonstration, for mutational occurrence and the driver-capacity, is the situation today. Unfortunately, this wrong-full dogma, is seen especially, in cancer research designed to facilitate development of therapy drugs (Precision Medicine Initiative) [38], and also ongoing in the very important work on re-activation of patients' immune system for "self-inflicted" tumor destruction. Some 50 mountain mutations, are presently in tests for immune competence, seeking FDA approval for patient trials [39] [40]. One article report: "-Major inter-national projects are underway-", -to construct a catalogue "-of all the genes responsible for the initiation and progression in cancer-" [41], based on the present day wrongful dogma. The mountain and other such mutations no doubt have "cancer" value, but how and why is unknown. There are however, some suggestions apart from the mutation theory which proposes a mutator mechanism, producing base pair deletions in repetitive satellite DNAs with repair-instability affecting mutational change of near-by protein coding genes (see below). Thus the "how" is the chromosomal "location-location" of the coding mutational effected genes, which becomes a non-random genomic mutational occurrence by the "linkage" to deficiencies in satellite DNAs. The other issue of "cancer value" of these different coding gene mutations, is presently hinted to from so-called "aneugenic" loss of heterozygosity (LOH) in tumor evolution, a rather frequently mentioned phenomenon in the cancer literature. This LOH suggested to be from "loss" of tumor suppressor genes is hinted to be from homozygous mutant alleles, haploinsufficiency and nullosomie loss of functional activity (−/−, −/0, 0/0) (in prep). In 1993 Therman and Susman wrote that ma-

lignant transformation occurred when the mutant allele is homozygous or both copies are lost (see below). The latter condition, is rather familiar from FISH analyses (fluorescent in situ hybridization), showing loss of whole chromosomes, arms and defined regions, obviously leading to loss of function from multiple genes. This fact may be part of the reason for observations of multiple cells (20 - 30) in cancers showing same, very complex karyotypes with several marker chromosomes put together from different chromosome bits and pieces, which was likened to speciation [42] [43] [44] [45]. These same karyotype cells, demonstrate functional genetic unity, most likely achieved by "extensive" LOH, and not from mutation theory basic assumption of cancer progression being caused by ongoing mutational-gain. Perhaps some of the enormous cost in cataloging these "mountain" mutations [41] [46] with questionable value, can be delegated to research into the meaning of LOH gain in cancer evolution.

4.2. Cells with Normal Karyotypes Are Not a Guarantee of a Normal Genome

Another dogma of normal karyotypes demonstrating normal cells, is still a valid belief, hard to understand in this day and age emphasis on mutations in cancer development. This dogma started with the detection of oncogenes (RAS/MYC) [47] [48], frequently found in tumors. Their "cancer importance" was shown in a bladder carcinoma experiment, in which the mutated RAS gene was isolated, and introduced into mouse 3T3 cells, because of normal karyotype, assumed to be normal mouse cells [49]. The conclusion was: "-that transfer of a single gene from cancer cells sufficed to transform the latter", which was "-transform of fully normal cells into tumor cells-" [49]. The claim of "-fully normal cells" is wrong, because as said, a normal karyotype, does not guaranty against possession of invisible molecular mutations. The 3T3 mouse cells had been developed under an NIH program for the study of the oncogenic transformation process to immortal "cancer" cells [50]. These cells and human 3T3 cells with gained fitness, were certified cell lines, and are listed as such in the ATCC Catalog of Cell Lines. In this ATCC Catalogue one 3T3-Swiss albino, mouse cell line, is described of being from disaggregated Swiss mouse embryo. The labelling of these various cell lines, 3T3, 10T10, 6T10, only meant the method (seeding density) by which they were produced, and has been a lifetime interest for studies of the transformation process by H. Rubin [51]. In essence the methodology of their derivations was nutritional deficit, which also was used in our establishment of the marsupial PtK-1 & 2 cell lines with normal karyotypes, and expression of multi, generational gain in fitness [52], they are also ATCC-listed as cell lines. In one PtK-1 experiment, 4n cells were shown to undergo genome reductive divisions from "amitosis", showing microtubules not "coming to a point", and that centrosomes were observed missing [53]. Claims of tumorigenesis from direct, normal cell-change to oncogenic transformed cells, is claimed from chromothripsis and multipolar division, but falsely, because these chromosomal "violent" processes

cannot be survival tolerated in normal human cells [3] [16].

Since the MT got a much needed support from the bladder RAS experiment, the believers are quick to refer to this event whenever the MT is threatened by other mechanisms for tumorigenesis, especially aneuploidy from the Duesberg laboratory. These scientists must have forgotten their own failed tumorigenic promises a decade ago [54] [55], and any new cancer-solutions for the myriad of mutations in cancer, has not been forthcoming, in spite of their continued sequencing identifications, to what avail? To this problem there is another difficulty, fast creeping into the cancer literature the word NEOSIS. This meaningless word, was coined from experiments with mouse/human 3T3 cells, believed to be normal cells [56], which certainly should be erased from cancer literature to come.

4.3. Cancer Cells from Normal Hematological Cells through 4n-Skewed-Division System

Primary requirement: *DNA genome damage in normal hematological cells*

The gateway to cancer differ for solid and hematological occurrences: for solid cancers the main issue is cell-freedom from cell contact inhibition, whereas for nomadic blood and lymphatic cancers, the big question is how such bone marrow, nomadic cells gain genomic damage, not yet clarified for the prevalent presence of translocations in such cancers [43] [44]. These abnormalities require DSBs on different chromosomes repairing by reciprocal NHEJ, and strangely the "breakpoint junctions" showed deletions (hundreds of base pairs) "at or near repetitive elements, especially *Alu* elements" (see below) [57]. The goal here is to gather evidence for a genome damaging model that can result in the 4n-skewed division-system, giving rise to fitness-gained, diploid cells with translocations. These reciprocal translocations showed specificity in involved chromosomes and the locations of the breaks, meaning that their derivations were not a random process [43] [44]. A possible answer to this intriguing observation comes from three reports on cells moving in marrow-like substrate, a meshed consistence of fibers and cells resulting in holes that moving cells would go in and out of [58] [59] [60]. The authors showed that human solid cancer cells moving in this artificial constructed substrate, suffered nuclear membrane rifts, which could penetrate to underlying chromosomal chromatin, and cause damage also to the underlying chromatin (chromatin leakage into the cytoplasm). A main conclusion was that: "-Nuclear integrity is temporarily compromised to accommodate cell migration-". Migratory cells in this substrate as mentioned, moved through narrow "squeeze-through" places. But essential for specific chromosomes to suffer damage was the innate, axial orientation of the nucleus in moving cells, which also gave rise to the breaks being specific, chromosome-location "pin-pointed". This karyotype pinpointing of breaks can on the molecular level be within megabits of DNA structure, because of interphase cells with a chromatin structure. Moreover, the authors found that cancer cells with migratory ability from dif-

ferent tumors, expressed differences in nuclear membrane stiffness, which could increased chromatin damage. The nuclear reduced stiffness, was found to be linked to abnormalities in laminar proteins, which normally cotes the inner, nuclear membrane. For example, in some cases, A-type laminar protein was absent, in other cells the amounts were reduced, which was seen as causative of less-stiff nuclear membranes, and more rifts. They also tested migration of one type of hematological cancer cells, and found nuclear rift-response.

4.4. Application of the above Findings to Nomadic Blood and Lymphatic Cells in the Marrow

These rather challenging observations, we suggest, can have happenings for hematological nomadic cells moving normally in the bone marrow substrate. Its consistency has also been found to be a mesh-work, from fat- and other cells in-between collagen fibers [61]. Thus, there is provision for existence of narrow squeeze-through places that the hematological normal, nomadic cells can encounter. The intriguing suggestion of specificity in chromosome and break occurrence is explainable from the fact that chromosomes are located in nuclear domains, which are in a permanent 3-D relationship to one another [62] [63] [64]. Provided, that the normal blood and lymphatic cells while moving in the marrow, maintain a steady, nuclear axial orientation, meaning that different individuals can suffer same chromosome with same break-locations. This then becomes a possible explanation for the observed specificity in chromosome and their break-locations observed repeatedly in different cancer patients [43] [44]. Another fact is that primary lymphomas have higher frequencies of translocations compared to leukemia (43). Could this be a reflection of lymphoma cells, being somewhat larger than blood cells, and therefore incurring more rifts? But most importantly is the new information of cancer cells expressing laminar protein abnormality, not only for hematological cancer initiation, but also for any type of cancer origin. This discovery opens-up practical/hands-on investigations never before a possibility in hematological cancer research, which even today is largely done by karyotype analyses for diagnosis, therapy and prognosis [65] [66]. To this, laminar protein practical approach, there is also hints of the marrow substrate, being denser in some individuals [67], which has been suggested as a possible inherent precondition [68]. All together there are now multiple reasons for the following question in analogy of babies born with immature lung developments: do children with leukemia-development at young age (also newborns), show marrow structural and/or laminar protein abnormalities, which can correct itself with increased age, if the child survives the harsh chemo-drug treatment?

5. Fitness-Gain from 4n-Skewed Division or from Leukemogenic Activity of Translocation Fusion Proteins

For decades break-repair to translocations has been interpreted to confer leukemogenesis (fitness-gain) from translocation derived fusion proteins. Leukemogene-

sis has been defined as: "-a factor that is known or attributed to be the cause of leukemia and progression of leukemic disease-" (Stedman Medical Dictionary). The ubiquitous translocation, t(9;22) (9q31;q11), in chronic myelogenous leukemia, has received the most attention as a leukemogen [43]. This translocation fusion protein is composed of partial protein coding DNA from the ABL gene on chromosome #9 fused to a part of the BCR gene on chromosome #22 [43] [44]. It has never been proven experimentally that this fusion protein is causative of leukemogenic increase in cell proliferation increase, compared to proliferation rate in normal cells. Therefore, the diplochro-mosomal 4n-skewed division system is tentatively accepted to become initiated from nuclear rift-associated chromosome damage. If true, this special division system to fitness-gained, diploid progeny cells would replace the rather debated leukemogen fusion protein with molecular mutations for fitness-gain. This proposition is supported by observation of diplochromosomes in untreated leukemia [69], which is likely to be observed again, if metaphases from patient's cancer cells are not treated with chromosome spreading solutions.

6. Mutated Protein Coding Genes with Locations on Borders to Satellite DNAs in Hematological Cancers

In the richly informative book on hematological cancers by Heim and Mitelman [43] there is mentioning of the ABL and BCR genes, being chromosomally located close to non-coding satellite DNAs, the chromosomal common fragile sites, the shorter length, mismatch repair sequences, and the telomere and centric DNA regions. The mutated ABL gene is bordering fragile site 9q31 and BCR is located at 22q1,1 almost in centric #22 heterochromatin in agreement with *Alu* chromosomal regions being deletion effected [57]. Putting these in vivo data together with known instability of chromosome fragile sites, induced from chemical agents or DNA damage, which can stress/delay the genome replication cycle, a theory for genome instability/mutator mechanism emerges [1]. The Glover, Oxford laboratory has for many years reported on replication stress from chemical agents or genome damage, effecting slowness and stalling of replication forks [70]. They found that such treatments gave rise to un-replicated lesions in satellite DNAs, the late replicating DNA. They have specifically focused on a "tight" relationship between deletions in the AT rich sequences, the common (you and me) fragile sites, located in (close-close) chromosomal fragility places. Over 100 FRA-sites have been mapped to chromosome specific locations, distributed over the complete haploid genome. These deletions in repair processes, have been found to cause genome instability from repair being inaccurate within "circumscribed" nucleotide regions, apparent in cloned, break site analyses [43]. The finding is that these repair processes cause mutations of structural genes, chromosomally located on borders of these repairing satellite DNA lesions (see below). This means that some structural gene mutations are not random events, but become mutated from a "bad luck" chromosomal loca-

tion, not the MT assumed way.

7. G-Banded Chromosomes Place Structural Genes in Dark Bands and Satellite DNAs in White Bands

Interestingly, G-banded chromosomes with white and darkly staining bands, giving individuality to the 23 chromosomes, were found to harbor structural genes in the dark bands, and that satellite DNAs were the unstained white bands [71]. A follow from this interesting fact, is that the length of the chromosomes upon banding procedure, will increase the definition of the bands, *i.e.*, high resolution banding. An example of this potential problem, is that average banded chromosomes will not reveal the small white band that is bordering the CDKN2A gene, the tumor frequent p16ink4a mutation on 9p21, it requires high resolution banding. Hence, in addition to the AT rich sequences in fragile site instability, the other three types of repetitive DNAs also reside in unstained white bands. Various pre-cancers (hyperplasia) showed repair foci on chromosomes where the mismatch repair sequences were located [72].

8. Repair of Unreplicated Deletions in Satellite DNAs

As mentioned above, deletion type nucleotide change will automatically seek repair process of which there are several types [73] that can cause diverse genomic alterations. For example, sister strand recombination with anaphase bridges in mitosis [74], can be causative of breakage-fusion-bridge (B-F-B) cycles, which is a frequent occurrence in cancers [75] [76]. Herein however, the focus is on the subtle interphase, repair process at max, involving 1000 deleted nucleotides [77]. Such repair is increasingly being found to be associated with aneuploid, copy number variance/-alteration (CNV/CNA) and structural gene mutations [78] [79] [80] [81] [82]. We see the molecular CNVs from gene duplications as "forerunners" of well-known gene amplified, microscopic visible homogeneously staining regions (HSRs) and or double minutes (DMs), also from single gene duplications [71]. In this book a chapter describes basic features of these chromosomally abnormal structures found in all cancers with the acceptance that sub-microscopic smaller duplications (CNVs) could extend length to HSRs. This thorough review, based on 26 specific references (6 referenced here) the occurrence and function in cancer cells is of special interest here. The classical work on HSR associated with cells being resistant to methotrexate treatment targeted to dihydrofolate reductase gene [83] [84] led to the suggestion of HSRs being the reason for chemo-therapeutic single and multi-drug resistance, and their origins from "saltatory replication" in a single S-period. The resistance to chemotherapy was explained by the amplified genes, 100 or more, being normal active (coding proteins), which "obviously" would be difficult to silence by for example chemo-drug methotrexate. Interestingly, 8 of the then known 30 oncogenes were found in HSRs or in DMs in cancers, and the MYC family of genes were examples of being "amplified in a whole spectrum of malignant diseases". HSRs and

DMs were proven inter-changeably in cells, meaning when HSRs disappeared DMs appeared and conversely [85] [86] [87] [88]. These latter reports on amplified c-MYC in HSRs or DMs, was suggested to initiate tumorigenesis, which should be applied to oncogenic transformation, and not to beginnings in normal diploid cells (pre-cancers), because of survival intolerance of chromosomal aberrations [16]. There are however, data indicating that molecular HSRs (CNVs) can be present in initiating fitness-gained cells, from probability occurrence in the ontogeny process of 4n-skewed division system (**Table 1**, **Table 2**).

1) Inactivation of Genes in CNVs and of "Mountain" Mutations

The stated normal oncogene gene expressions in HSRs (DMs), has been challenged, by words of "elusion" from expression or of the genes being in a "latent state". But since these interpretations have been debated, they are not referred to here. However, undebatable proof of gene inactivation from duplications is known from *Drosophila melanogaster* genetics [11]. In this organism genetics for normal eye-size has been localized to one dark band in salivary chromosomes (polytene chromosomes), and the dominant BAR-eye phenotype, showed two bands with result of the round eye reduced in size by a kidney-shape. To this condition, a third band was introduced by recombination techniques, and the result was the Ultra Bar-eye phenotype with almost nothing left of the compound eye structure, decidedly showing *genetic inactivation*. No doubt several genes determine the development of the normal eye-size, but when these genes were duplicated/triplicated, *the genetic system for eye-size became functionally inactivated*. In one work on cancer CNVs [82], there is claim of associated "evasion" of the immune system, here interpreted to mean *functional inactivation of immune-determining genetics*. Importantly, this is not the only gene inactivation system, it also occurs from so-called position effect (PE), shown in *Drosophila* [11] to be from structural, coding genes coming in close contact to heterochromatin. Note, when genes located close to repairing satellite DNAs (forms of heterochromatin), and become mutated (mountain mutations) from repair of such deficiency lesions, the normal location of this gene becomes closer to heterochromatin for *PE functional inactivation effects on these genes.* This because micro-deletions are created in these unstable repair processes, and can be causative of a closer associations between the mutated gene and heterochromatin [43] [70]. In this regard the evolutionist "chromosome-thinker" Yunis, [89] proposed that segments of duplicated nucleotide regions, which were types of heterochromatin, provided structural stability to chromosomes. In this, we note, that present satellite DNAs are forms of CNVs devoid of genetic activity. Can this mean that the mechanism for cancer-associated duplication, causal of gene inactivation, is conserved in our genomes?

2) If PE-heterochromatic inactivation of the most frequently mutated genes identified in tumor sequencing work, is true, and there also is inactivation of genes from duplications (CNVs), then present concepts for therapy and chemo-drug developments, will have to be drastically changed. With truism, the

expectation of present chemo-drug treatments, specifically targeted to active cancer mutated genes (tailor-made treatment), will have dubious, positive prognostic effects, which is true, except for treatments of children's leukemic cancers with eradication of some types. This lack of a positive response is unfortunately based on the belief that mountain mutations confer fitness-gain upon cells, and therefore tumor driver capacity, which completely lack evidential proofs [1]. The original discoverers of mountain mutations, themselves stated that those characteristics were ideas, unproven in cell cultures by them [35] [36] [37] [94]. Why is it that this therapy destructive dogma cannot be accepted even by "officers" in charge of federal funding, and with decisions of what aspects of the cancer mystery, should be pursued. Going back to the discovery of CNVs being correlated to immune "evasion", it is very significant that these events were also correlated to reduced response to immunotherapy [82]. Yes indeed, because the genes in the CNVs were functionally inactivated, which might mean not producing gene-product and therefore, not targetable by immunotherapy. Furthermore, it may well be that mountain mutations inactivated by PE, also do not produce therapy targetable proteins, which suggest that the lack of normal gene function is the culprit in some cases of cell damage. The APC protein even with presence in cells, the cellular effects are better understood for the lack of normal function ([1], see APC gene). APC gene is also chromosomally located in fragile site repetitive DNA at 5q21, and therefore has a *bad-luck chromosomal location for occurrence of the mutation and its PE inactivation.* In all of this it is clear that present ongoing mutation-discovery works [38] [41], including development of chemo-drugs with specific action to these mutated genes' actions needs reconsiderations.

9. More Information Is Needed for Therapy Combat of CNVs, HSRs & DMs

The HSRs and DMs are "only" known from tumors, whereas the shorter, molecular CNVs (HSRs) have been produced in relationship to genomic damage cells [90]. All three gene duplication structures are from Drosophila genetics considered to be originated by saltatory replications [86], expressed by unequal crossing over [88]. This origin of visual HSRs can be studied in normal human cells from our observation of duplications on chromosome #7 in cells exposed to SV-40 virus [91]. These structures on the p-arm however, were not homogeneously staining, the normal terminal dark band (G-banding), was duplicated with a light band in between to various lengths (1 to 3 - 4 dark bands) HSRs in different cells. This suggestion of in vitro study also for molecular CNV duplications [90], is becoming a reality demand, because the medical significance for therapy, has become many-fold increased. This is because there are now three (not only CNVs) different chromosomal structures possible to be formed from the same mechanism of origin. If as proposed CNVs, HSRs and DMs are gene inactivating mechanisms as proposed from Drosophila Bar-eye phenotype cyto-

genetics [11], then expectation is that not only oncogenes, but also other structural genes in bad-luck chromosomal locations will be identified in these various type duplications. With this increase in quantity and assorted involvement of genes in these structures, it may well be that they will be found to be the real drivers of tumorigenesis. We repeat: "-It is reasonable to *assume* (our emphasis) that genes that are mutated more often than predicted by chance are more likely to be drivers-" [35] [36]. The present fallacy of immunotherapy targeting the "mountain" mutated genes (39, 40), is shameful misuse of optimistic patients. This ongoing costly work for therapy, should be redirected to investigations into chemical pathways in the mechanism(s) leading to gene duplications. No doubt, informative knowledge can be gained by molecular identification(s) on likely genome conserved "driver motor" for successive gene doubling process. The how(s) of this event, might prove to be a major discovery for effective therapy treatment. And, from the knowledge that both SV-40 virus infected and X-ray damage cells respectively responded with visual HSRs and CNVs, the foundation for experimental enhancing manipulations is already at hand.

Interestingly, in Bloom's cancer syndrome, chromosome damage by breakage preceded occurrence of high rate recombination processes [71]. But the intriguing fact in this cancer prone disease, was that this recombination occurred in tetraploid diplochromosomal cells, a discovery earlier detailed [26]. This brings into this discussion, ontogeny of the 4n-skewed division system in the origin of CNVs, which now can be suspected of capacity for visual "HSRs" (#7p-arm). Thus, there are already several avenues known for the unraveling of the mechanism(s) involved in the origin of these cancer likely driving factors. This latter characteristic is concluded from their ontogeny being associated with the 4n-skewed division system, and their presence in the malignant phase. The Oxford laboratory concluded that the mechanism for mutation of structural genes and for CNVs was different [79] [89]. Altogether, there might now be hopes for cancer vaccine development [92] achieving eradication of cancer disease, which was promised a decade ago, but remarkably, no-longer mentioned, why? Cynically, such development is doomed unwanted, because of anticipated piles of money to be made by keeping the cancer mystery alive [39] [40], which will drive the prices for treatment becoming more sky-rocketing than now, singling treatment to the rich.

10. Mutation or LOH of Coding Genes Located on White-Dark G-Banded Chromosome Borders

The well described tumor suppressor Fhit gene with location "in" fragile site 3p14.2, is an example of a structural gene being mutational effected by deletion within the "FRA3B/FHIT locus" [93] [94]. Another gene Ptch with location bordering FRA9D-9q22.3, and the highly malignant Burkitt's lymphoma translocation t(8;14) with breakpoints in fragile sites 8q24 the location for c-myc, and 14q32.3 the place for the immunoglobulin heavy chain (V). C-myc gets lost and

replaced by the V genes. These and the above mentioned locations of mutated ABL and BCR genes on borders to satellite DNAs spurred the question of whether chromosomal locations of other frequently mutated genes were closely situated to unstable satellite DNA lesions? Since the chromosomal locations were from numerous different publications, references are not cited here. The p53, Rb, APC, p16ink4a genes, were found with the respective locations: 17p1.1, 13q14, 5q21, 9p21, all on dark chromosome band's borders to satellite DNAs. These observations became more meaningful from the oncogenes MYC-N, MYC-C and RAS, located at 2p24.3, 8q24, 11p15, also corresponding to white-dark band borders. The conclusion was that chance was not operating in the mutation process of these genes. They were a happening from bad-luck chromosomal location. In a way, this fact is supported by twice as many mutations in dark DNAs as compared to coding gene mutations reported from sequencing works of tumors [95]. The following **Table 3** with chromosome locations of tumor suppressor genes was also from irrelevant literature, and speaks for itself.

Table 3. Mutated protein coding genes in tumors.

Affected gene	Chromosome location	Fragile site
HlC-l	17p13.1	also location of TP53
ST7/RAY l	7q31	FRA7G-q31
SEZ6L	22q12.1	FRA22B-q12.2
RASSFlA	3p21.3?	FRA3A-p24.2
ARHl/NOEY2	1p31	FRA1C-p31.2
TSLCl	11q23.2	FRA11G-q23.3
M6P/LGF2r	6q26	FRA6E-q26
PPP2RlB	11q23	FRA11G-q23

11. Molecular Genome Sequencing of hematological Cancers

Specifically for hematological diseases, uniparental disomy (UPD), has been discovered with high frequencies [96] [97]. These authors, by SNP analyses identified what they called copy neutral (CN) loss of heterozygosity (CN-LOH) with the most often involved mutated genes being *JAK*2, *MPL*, *c-KIT*, *and FLT*3. Whether these mutations are consequences from repair-associated recombination events in satellite DNAs is not known, since their chromosome locations were not given. Another SNP analysis of 242 pediatric acute lymphoblastic leukemia (ALL) [98] showed that seven specific mutations had repeated occurrence in different patients, and that these mutations were: TP53, RBI, FHIT, CCNC, CDKN2A (p16-ink4a), MLLT3-(ETV6), ATM (MET?), which the authors, themselves, placed nearby satellite DNAs. Interestingly, the most frequently mutated genes in solid tumors, p53, Rb, p16-ink4a and FHIT, were also mutated in hematological cancers, which favors involvement of the 4n-skewed division-system. Another such SNP analysis of one case of acute myelogenous leukemia (AML),

showed ten different satellite DNA anomalies [68] in agreement with the latter ALL observations. A long awaited therapy improvement, is that these mutational identifications, are beginning to influence therapeutic approaches for instance, by targeting the frequent TET2, ten-eleven translocation in myeloid and lymphoid cancers [99]. Interestingly, this tumor suppressor mutation, is a loss of function mutation from either deletion (0/0) or inactivation (*Drosophila* above), but could also be from mutation leading to haploinsufficiency (−/0, +/0). The physiologic pathway in the TET2 promoted pathogenesis (different from type of mutations?) is an important route to likely several therapeutic drug possibilities. Unknown in all of this information is however, the most important question: how these malignancies were initiated. Tentatively we propose that some cells with nuclear rift-caused genomic damage, and can repair while the cell continues replication [70] [90] with outcome of the 4n-skewed division system, and its multiple consequences [100] (**Table 1** & **Table 2**).

12. Haploidization in Hematological Cancer Cell Origins

Near-haploidization from separations of maternal and paternal genomes (mat/pat) is a reality mechanism for tumorigenesis in both solid and hematological cancers. Why? One reason is that this type of haploid genome segregation, called gonomery in some *Crustacea* orders [101] are likely conserved in our genomes [102] [103], and consists of maternal and paternal genomes segregating from each other. This great loss of tumor suppressor genes (aneugenic LOH), allows near-haploid and/or hyper-diploid, uniparental greatly homozygous disomic cells to proliferate unhindered. It is a very rarer occurrence, believed to occur in both solid and hematological disorders (e.g., solid cancer: small cell lung cancer). Below some examples of haploid, leukemia cells are listed (**Table 4**).

Table 4. Near haploid genomes in different types of leukemia.

26, XX, t(9:22)(q34:q11), -1, -2, -3, -4, -5. -6, -7, -9, -10, -11, -12, -13, -14, -15, -16, -17, -18, -19, -20. –
27, X, -Y, t(9:22)(q34:q11), -1, -2, -3, -4, -5, -6, -7, -9, -11, -13, -14, -15, -16, -17, -18, -19, -20, -22. –
28, XY, t(9:22)(q34:q11), -1, -2, -3, -4, -5, -6, -7. -8, -9, -10, -11, -12, -13, -16, -17, -18, -19, -20.
28, X, t(9:22)(q34:q11), -X, -1, -2, -3, -4, -5, -6, -7, -9, -10, -t(11:?)(p?:?), -12, -13, -14, -15, -16, -17, -18, -19, -20

Comments: the t(9:22) translocation is also an almost stable occurrence in different patients with CML, and as here for near-haploidization with remarkable, repeated same breakpoints (q34;q11). This simple translocation can become complex with 3 - 4 different chromosome involvement: t(2;9;22)-(q23;q34;q11), t(5;9;22)(q35;q34;q11), t(9;15;22) (q34;q11;q11), t(8;9;12;22)-(p21;q34;q21;q11) with break-points in added chromosome being same or different in different patients [44]. (The likely mechanism behind these multi-chromosomal translocations, is a subject unpublished.)

These **Table 4**, rather impressive karyotypes with almost complete loss of one complement, has the explanation of origin from non-disjunction, but diploid yeast cell-strains (*S. cerevisiae*) offers another explanation [104]. These different strains contained different deficiency lesions, and as a result showed high rates of sister strand recombination repair, which gave rise to excessive, genomic in-

stability. It was suggested that as a consequence of this instability, the genome "rescued" itself by haploidization. Interestingly, we observed a somewhat similar haploidization process in normal cell populations made to carry genomic damage [22] [23] [24]. Several of the diploid cells segregated near-haploid cells, and cells in the population showed bizarre genomic aberrations, such as under-replicated region (single strands) and genome instability by random chromosome breakage into bits and pieces. These about 40 photographically illustrated abnormalities were all from 2n/4C cells, consistent with sister strand recombination events. In addition the process of near-haploidization was supported by a table listing chromosome numbers at 23 and the spread of deviating numbers, surprisingly limited to only 6 ($17 < 23 > 29$). These various anomalies are in full agreement with the yeast cell observations, and completely support the suggestion of high genomic instability, being a trigger of the haploidization event, a suggestion needing conformational proofs for human cells. This separation of maternal and paternal genomes, showed the consequence of 1/2 reduction in cell volume compared to normal diploid cells, which even with change to diploidy retained the "small-cell" phenotype. This naming is used in hyperdiploid leukemic cells [43], and as mentioned in small-cell solid cancers, which should be analyzed for extent of homozygosity. In gynecologic cancer-linked pathology, the small-cells were measured by 1/2 volume reduced [105].

Hence, in hematological cancer initiation there are now two possible routes for cancer initiation, the mechanisms from 4n-skewed division-system and from gonomeric haploidization. In both of these processes the suggestion of origin, is from marrow-substrate with "squeeze-through" narrow places, causing specificity in targeted chromosome with break/deficiency in same locations. These long known phenomena, expressed by multiple patients, could be explained by permanent nuclear placement of chromosome domains relative to each other, while going through the narrow, marrow places. If true, leukemia/lymphoma reported translocation chromosomes should be in domains close to each other for broken ends to find each other, which "domain-enthusiasts" can answer. For example, for the ubiquitous (9;22) translocation, are the domains for chromosomes 9 and 22 close to each other for both chromosomes to suffer breaks in one squeeze-through place? Another unclear situation is how multiple breaks occur in marrow moves to give rise to high recombination rate for the triggering of near-haploidization. This rarer process, but prognostic very poor, especially in children, may develop from smaller than normal-size, squeeze-through holes in the marrow, from inborn (inherited) denser/thicker marrow substrate as seen in aging [67]. Inheritance may also apply to laminar protein anomalies, which from "softer" nuclear membranes would be vulnerable of multiple membrane-rifts, hitting different chromosomal domains with breakage. These questions and suggestions can get answers from practical/hands-on investigations of marrow substrate and laminar protein compositions in today's patients. But only possible, if the *will* and *money* can be diverted from questionable tumor sequencing [41]. If so, Vogelstein's wish of closing children's cancer wards [106] may be fulfilled, a wish we all share.

13. Conclusions

A rather broad field within cancer occurrence has been covered rather detailed from own experimental data on note, normal human cells and from various pro/con literature reported data. Since conclusions and suggestions of *what to do next* have been stated, it is not repeated here. We do however, stress that diplochromosome, 4n-skewed division-system, has gained more experimental evidential facts, supporting our proposed role in (some) cancer initiation and evolution. The second point is that our observations (pictures) of this diplochromosomal tetraploidy, giving rise to fitness-gained karyotype normal diploid cells, have "historic" simulants, listed in the ACCT Catalogue of Cell Lines as 3T3 & 10T10 human and mouse, karyotype normal cell lines, not yet oncogenic transformed. This was an NIH project for the unraveling of the oncogenic process, because only benign tumors (diploid hyperplasia) had been observed to give rise to dysplastic tumors with immortality and activated telomerase gene. From these and other data (below) the conclusion is that cancer tumor progressive development, is generated from cell constitutive inheritance, which from tumor infancy (initiation) becomes a competition between genetic varied clones, endowed with the main characteristics of environmental selected proliferative capacity and resistance to the immune system and apoptosis/necrosis events. This clonal competition is a reason for many therapy failures, because single-drug therapy instead of multi-drug treatment was used. And there is also the possibility of near-time staggering of different treatments, but in all of these treatments it should be known if the drug can operate as a carcinogen, here shown to lead to 4n-diplochromosomal cells with its mutator phenotype (see text). But best of course, is prevention of initiation, which has great likelihood of being achieved by identification of molecular drug targets in the ontogeny process of the 4n-skewed division system. NOTE, this is an only mechanism known today in over a century of cancer research to confer generational gain of fitness upon normal human cells where cancer starts.

In the past studies, we considered solid cancer development from the amitotic, 4n-skewed division-system, which herein was concluded to be linked to instability of fragile sites and other repetitive satellite DNAs. Furthermore, a comprehensive assessment of relevant cancer literature, concluded: 1) that mutations of protein coding genes, included frequent and less frequent tumor suppressor genes that could be allele-wise present as 0/0, −/0, +/−, deletion and haploinsufficient genomic functional; 2) a *new discovery* was that the high frequency mutations identified in tumors, were chromosome located on G-banded, dark band's borders to satellite DNAs (fragile sites, mismatch repair repetitive DNA, telomere repetitive regions and heterochromatic centric DNA); 3) these chromosome regions when affected by deficiency lesions, performing repair process in both solid and hematological cancers could be causative of both mutations in structural genes and produce CNVs; 4) these CNVs were concluded to be molecular expressions of long-known HSRs and DMs, observed in cancer cells; and 5) the assumed active oncogene expressions from these gene amplifications, con-

ferring resistance to cancer therapy treatment, were seriously challenged (being wrong) by the fact that *Drosophila cytogenetics* had demonstrated *genetic inactivation from duplications*; 6) introduced was also heterochromatin associated PE, also known from *Drosophila*, to *inactivate genes* with locations near-by; 7) this latter situation is a high probability for the cancer frequently mutated genes, when satellite DNAs is considered different forms of heterochromatin [see 2) above], and 8) these new findings and consequences will have dramatic effects on present approaches in cancer therapy, which today adheres to the mutation theory. But, change is necessary, considering highly likely *genetic inactivation in tumorigenesis*. Then, the big question is how to therapy treat oncogene-negative, functional expressions?

In hematological cancer development, the special issue is how normal nomadic cells in the bone marrow attain genomic damage, especially DSBs in specific chromosomes to construct multi-patient occurrence of same translocations rather prominent in these cancers. Three resent articles described cancer cell movement in artificial marrow-like substrate, with information on: 1) narrow cell squeeze-through places in the substrate, causative of nuclear "rifts" penetrating to underlying chromosomal domain with breakage, and 2) the constant nuclear location of the domains relative to each other could explain specificity in chromosomes involved and their same break-locations, observed from translocation analyses. But this breakage phenomenon in normal cells also satisfied the required genomic damage for presence of the 4n-skewed division-system. This existence would replace un-proven leukemogenesis from translocation fusion proteins with molecular mutations. A hitherto unknown observation in the artificial substrate was that some mowing cells showed laminar protein anomalies, which reduced nuclear stiffness, and increased severity of chromosome damage. Lastly, these diseases showed SNP analysis, revealing deficiency lesions in satellite DNAs, and that the mutated structural genes, were the same high frequency genes as reported in solid cancer development. Thus therapy, is in the same dilemma as for solid cancers (see text). But one complaint is that the wording: "copy neutral loss of heterozygosity" does not convey a genetic or functional meaning. But from the fact that the condition can exist as an UPD, we interpret the wordings to mean: segmental haploid loss of heterozygosity (LOH), quite often shown by FISH of tumor cells. Please cancer chemists, do not invent words for your biological findings (consult), it as in this case, can erase reference to already known cancer genomic change, and not be a "novel" discovery. With this in mind the artificial marrow-like substrate did open up practical/hands-on approaches for the gain of more information on crucial issues in the development of these diseases. But is the *will* and funding there? Yes, for the sake of closing children's cancer wards, and gain information for more effective treatment.

14. Flow-Chart of the Most Important References

The present author comes from *Drosophila* genetics with specialty in cytogenetics to cancer-genetics at a time when there were revolutionary discoveries in

DNA and chromosome structure, and in methodologies for the culture of normal human cells. Consequently, a well diverged book and individual publications are on-hand from this very rich period of cell biology in cancer, especially from the Therman, Yunis and the Glower laboratories. The present focus on diplochromosome tetraploidy (4n-skewed division-system) stems from this early time, which presently has been developed to the view of being a constant source of molecular alterations in cancer evolution, it has a mutator phenotype.

Effects From Specific References	References
-Diplochromosome tetraploidy occurrence:	[4] [5] [6] [7] [12] [13] [14] [15] [26] [33] [52] [69] [71]
-4n-skewed division-system (4n-SDS) and 90˚ nuclear turn:	[6] [7] [10] [12] [13] [14] [21] [30]
-Accepted provisions for cancer beginning from normal human cells:	[2] [3] [16]
-Cancer hallmark: gain of fitness, not in MT, but positive in 4n-SDS	[9] [22] [24] [28] [35] [36] [37] [45] [46] [51]
-Origin of genetics for fitness-gain, the ontogeny of 4n-SDS	[1] [4] [5] [6] [100]
-4n-SDS and fragile site (dark DNAs) deletion-repair = genome instability	[1] [27] [28] [43] [70] [71] [72] [79] [80] [81] [89] [90] [100]
-Consequences from the above in cancers:	
-Gross chromosomal: aberrations, B-F-B cycles, HSRs or DMs	[74] [75] [76] [83]-[88]
-Molecular: mutations of high frequency mutated genes (p53, Rb)	[1] [91] [93] [94] & herein
-Molecular *special*: copy number variance/alterations (CNVs/CNAs)	[77] [78] [79] [80] [81] [93] [94]
-Why special? Oncogene CNVs (HSRs) with assumed gain in gene activity from hundreds or more gene amplified is an explanation for their drug resistant therapy	[47] [48] [84] [87] [96]
-WRONG! *Drosophila* cytogenetics of salivary polytene chromosomes, demonstrate *inactivation of gene-function when duplicated* (see text books citing BAR phenotype)	[11] [82]
-In *new* therapy program, gene inactivation from PE [11] must also be considered, herein suggested to inactivate the high frequency mutations, because of their close location to satellite DNAs, which are forms of heterochromatin, the culprit in PE (see conclusion).	
Hematological cancers, dependent on genomic damage in normal cells:	[58] [59] [60] [61]
Location of nuclear domains relative to each other; special significance:	[62] [63] [64]
Presence of diplochromosomal tetraploidy (4n-SDS) in leukemia:	[69]
which replaces leukemogenesis with molecular mutations	
4n-SDS to same type molecular lesions and resultant mutations:	[68] [96] [98]
New discoveries: bone marrow density increase (?) and laminar protein anomalies:	[58] [59]
Same therapy questions (above), except for leukemic haploidization:	[22] [23] [24] [43] [44] [103]

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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Abbreviations

MSP = mitototic slippage process
DSB = double strand breakage
CNV = copy number variation
CNA = copy number alteration
GCIN = genome-chromosome instability
EMT = epithelial mesenchymal transition
HSRs = homogeneously staining regions
DMs = double minutes
MT = mutation theory
PE = position effect