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Viral and Nonviral Oncogene Theories of Cancer

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Abstract

Rather than writing a review with a limited scope, we attempted to survey the span of medical and molecular biology research in the field of oncology during the last six decades: from the early breakthroughs in viral oncology and the novel notions of virus and viral cancer (*onc*) genes; to the role of DNA cancer viruses and C retroviruses in experimental carcinogenesis; through the discovery of cellular oncogenes and the biology of growth factors; to the expansion of the concept of oncogene and its types; and, finally, to the realization that cancer is a multiplicity of different disorders that appear to arise through nonviral auto-oncogenic processes involving adaptive changes and epigenetic responses to cancer-promoting pressures in the external and internal environment of the organism. Throughout, we have searched for the integration of an oncogenic vector with different degrees of transformation, seeking the commonality of proliferative disorders, somatic cancer and leukemia. We re-examine what separates transformation, benign and malignant, from differentiation, and how their reversible switch deploys graded responses related to states of hypersensitivity to, or independence from, key physiological growth factors.

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> "In a virus-induced cancer, a normal cell is altered by an oncogenic virus. The malignant cell continues to grow and divide, and considered by itself is healthy. A cell, however, is not an independent unit but a dependent part of an organism. An organism controls the growth and multiplication of the normal cell but not of the malignant one, which behaves as an independent unit. Its multiplication causes the death of the organism. The oncogenic virus, although it only modifies a cell, kills the organism and is therefore pathogenic."
>
> A. Lwoff, 1958 [1]

1. Emergence of virology and research into the viral etiology of cancer: viruses vs living systems

The term "cancer" means 'crab' in Latin, and comes from the description of a tumor as a central mass that invades surrounding tissue with crab-like claws. Cancer has been known as a form of disease for at least 4,000 years, since the time of known papyri which describe cervical cancer and surgical trepanation for the removal of brain tumors. But the biology of cancer, ie oncology, is only barely a century old. It began in the form of a viral theory of cancer. Paradoxically, by the time that its development - spurned by the study of bacteriophage that infect bacteria - had led to a full molecular and biochemical elucidation of DNA and RNA viruses, and their involvement in infection and malignancy, most cancers could no longer be regarded as being viral in origin.

The notion that infection with viruses (from *virus*, Latin for 'poison' or 'venom') may cause cancer dates back to Peyton Rous discovery of the viral etiology of a malignant chicken tumor in 1911 [2], when he reproduced the sarcoma in chicken by injection of a cell-free filtrate [3]. R. Shoppe repeated the demonstration with a cell-free filtrate from a rabbit fibroma in 1932 [4]. These experiments suggested that at least the first three postulates of Koch could be confirmed: the agent was isolated from the pathological lesion, could be transmitted to a like host and, once transmitted, caused the same disease. Ludwik Gross went a step further in the 1950's, and showed that virus-induced fibrosarcomas in the newborn mice could be prevented by immunization of the mother, fulfilling Koch's 4th postulate. He also discovered that murine leukemia could be transmitted with a filtrate [5]. As detailed by Gross in a review of the findings from research in the 1950's, leukemic viruses could be isolated from spontaneous leukemias in high-leukemic inbred mice strains, from virus-induced leukemias in rodents, from radiation-induced leukemias in low-leukemic inbred mice strains and from transplanted mouse sarcomas and carcinomas [6].

Social resistance - in the form of peer-review rejection, media derision and unavailability of funds - to the modest beginnings of virology and oncology was nearly as intense as that met, nearly

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in parallel, by Reich's medical and 'bion' research in what he called "the cancer biopathy" [7]. Lwoff, Luria, Gross, and many others, suffered ignominious attacks. Gross, in particular, was the subject of a concerted *ad hominem* campaign [8], which eventually ceased when a new generation of researchers trained with molecular techniques began corroborating his findings.

A substantial component of the irrationality of the epoch was a consequence of the then ongoing revolution in the biological understanding of what is a virus and how it is distinguishable from an organism, be it a micro-organism. The definition of virus which has since remained was that magistrally legated by André Lwoff in the 1950's [9]. It is based on the notion that viruses are neither organisms, nor cells or mere molecules. Lwoff's accent lies in the definition of the living as an "organism", as "an independent unit of integrated and interdependent structures and functions" [9]. An independent existence requires: (1) an autonomous energy metabolism ("the presence of a Lipmann system") that permits growth; (2) an ability to autonomously replicate *in toto*, ie *replicate* not only their genomic DNA or genetic material but *reproduce* the entirety of their organellar, cellular, tissue and organic structures; and (3) the ability to *multiply*, whether by mitotic (self-)division and proliferation or by a combination of the latter with a form of propagation. Now note that Lwoff's requirements for an "independent existence" do not define the major trait of Lwoff's concept of an "organism". Instead, this trait emphasizes *the dependence of the parts on the whole* and *the interdependence of structures and functions in the organism*. This conceptualization is nearly parallel to Reich's definition of the organism as a system having *a unity of function and structure between very different organs*. Both Lwoff and Reich explicitly reject the notion that the organism is the sum of its parts. But it must be said that the concept of 'dependence' is too wide and therefore somewhat vague - as is the concept of independence or autonomy. One may grow cells from a metazoon outside of its system, in *in vitro* conditions where that dependence is abrogated - even if it is still residually manifested by the specificity of factor requirements. One may grow organelles or endosymbionts (eg dinoflagellates, etc) independently from the host cells, and so on. Moreover, no living system is ultimately independent of other living systems (as the concept of ecology well illustrates) or from the physico-chemical conditions of its environment (fluid and energy media). In this respect, Reich provided a better formulation of the concept of biological unity when he described the major trait of a living system as being provided by "the function of the whole in each individual part" [10]. It is not so much "the dependence of the part on the whole", as it is the fact that the whole functionally resides in each of the parts, in the form of a function of the part which is, in that part, already the function of the whole. The whole is a part apart from the parts, but it is composed of the parts of the whole in each of the parts. The unity of structure is merely a consequence of the unity of function that results from the consistent function of the whole in the functioning of each of the parts. But we should rather prefer to call

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'system' what Lwoff and Reich call an 'organism' - a 'discrete living system', it being understood that such a system is always open through energy and fluid (molecular) continuity with an outside. Similarly, the entirety of the requirements for autonomy - in energy metabolism, in morphogenesis as well as in replication and division - is a corollary of the self-ordering property of a living system, which is the expression of the capacity of the system to increase its internal energy by energy capture, conversion and accumulation [11].

Lwoff argued that metazoa, protozoa and prokaryotes all form organismic entities (living systems) - a cell in a metazoic system or a colonial lifeform being as much a dependent part as is an organelle in a protozoon [9]. An organelle - Lwoff suggested - does not have an independent existence, even if it once did (such as mitochondria). Nor do viruses, he stated. In fact, in the wake of Beijerinck and Bawden, Lwoff claimed that "it is clear that viruses have more in common with cellular organelles than with micro-organisms" [9]. As proof, he mentioned that both organelles and viruses present nucleic acid or genetic continuity (this observation only applies to DNA-carrying organelles) and depend upon the cellular metabolism of the host for their replication and multiplication. This is all the more provocative as Lwoff placed one more accent in the definition of a virus that he was providing: the ability to be infectious which, in his strict view, had to distinguish a virus from "all the normal cell structures which can penetrate into another cell" [9]. The distinguishing trait was "the introduction into an organism of a foreign entity able to multiply, to produce a disease, and to reproduce infectious entities" [9], with the term 'foreign' being *the connection to an outside* that ruled out the virus as being an organelle. Thus, we immediately remark the weakness of this distinction of a virus from any "dependent part" of a living system, and the petition of principle it incurs. If viruses are parallel to cellular organelles and do not exist or multiply independently from host cells, then one should conclude that their original creation must, in each instance, have been a biological production - a cellular emission of a "normal cell structure able to penetrate another cell". Before any original virus ever had the chance of being multiplied, it was a truly 'endogenous' virus. The concept of 'infection' as a linkage *to an outside* is a secondary characteristic, no matter how important, since it is effectively inseparable from the notion of 'production of a viral particle *from an inside*', by a living system. This weakness, however, remained the 'unspoken' of medical virology - which gives one the measure of contrast with Reich's "orgonomic" approach to the etiology of cancer, which claimed that cancer was *not infectious*, and instead was an *acquired*, *endogenous disorder*, where even the production of 'T-bacilli' (or mycoplasma [7]) was an *endogenous* (and 'heterogenic') production. Curiously, Lwoff actually addressed these problems in that magistral 1957 lecture. He proposed, in fact, what was a radically new concept of viruses, that they were *truly endogenous in origin*: "viruses (...) originated from some pathological constituents of their host cell" [9]. Using the bacteriophage as example, "the

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endogenous theory" (Lwoff) held - precisely as we just suggested above - that viruses originated in bacterial genes as dependent parts of the bacterial system: "The prophage is obviously not independent. It behaves as a dependent part of an organism" [9]. But in thus employing the term 'endogenous' to designate the origin of bacteriophage - and any other viruses by extension - as effectively heterogenic, Lwoff explicitly refused assimilation of 'endogenesis' to 'heterogenesis': "The inquisitors of faith have tried, and are still trying, to ridicule the endogenous theory by brandishing the threadbare scarecrow of heterogenesis" [9]. Yet, the only avenue left for Lwoff to take that could dissimilate the two concepts was not the one he chose - for 'endogenesis' of viruses could only be different from 'heterogenesis' to the extent that the latter could only be said of living systems and viruses clearly do not constitute living systems. However, when it comes to the biological ontogeny of viruses qua viruses, to say that they are endogenous is still to say that they are heterogenic, that they (viruses) arise from different and irreducible elements, from cells, from their host cells. Moreover, for all effects and purposes, this made any concept of infection secondary to the processes that, endogenously to a cell, heterogenically create a virus. And while it is apparent that viruses have the ability to infect in a manner analogous to bacteria and protozoa, this ability is a passive one, since - as Lwoff was well aware - host cellular susceptibility (permissiveness) and immunity are the ultimate determinant factors of whether an infection succeeds or not.

Irrespective of the shortcomings in Lwoff's definition of a virus, it is evident today that viruses constitute a unique category of biological entities that cannot be regarded as forming biological systems. They should, rather, be thought as dependent parts of biological systems. Viruses are not considered to be living systems, to constitute organisms or even cells, since they cannot autonomously replicate and multiply, and they lack metabolism. Viruses neither grow nor divide. Thus the existence of a virus does not require a source of energy, only its production and reproduction do. But for these 'purposes', every virus depends upon the protein and genetic machinery of a host cell that it must infect. Further, viruses can only be multiplied in the form of their nucleic acid. There are no known living systems whose genome is composed of RNA, but all living systems contain both DNA and RNA. Conversely, the genome of viruses may be composed of either DNA or RNA, and viruses only contain one type of nucleic acid - from which the virus may be multiplied. Viruses are therefore strictly parasitic molecular assemblages of proteins and genetic code fragments. They are not *living* in the cellular or systematic senses, but *alive* in the molecular sense that all viruses are biological productions, or nucleoproteinic *biomolecular constructs*. The key component of each virus is the genome, their genetic sequence, which may come in single or double stranded varieties of either DNA or RNA.

By the late 1950's, viruses and virus-like particles (see **Fig. 1**) were being isolated from a variety of somatic tumors and leukemias in animals, and from some human leukemias (even though lack-

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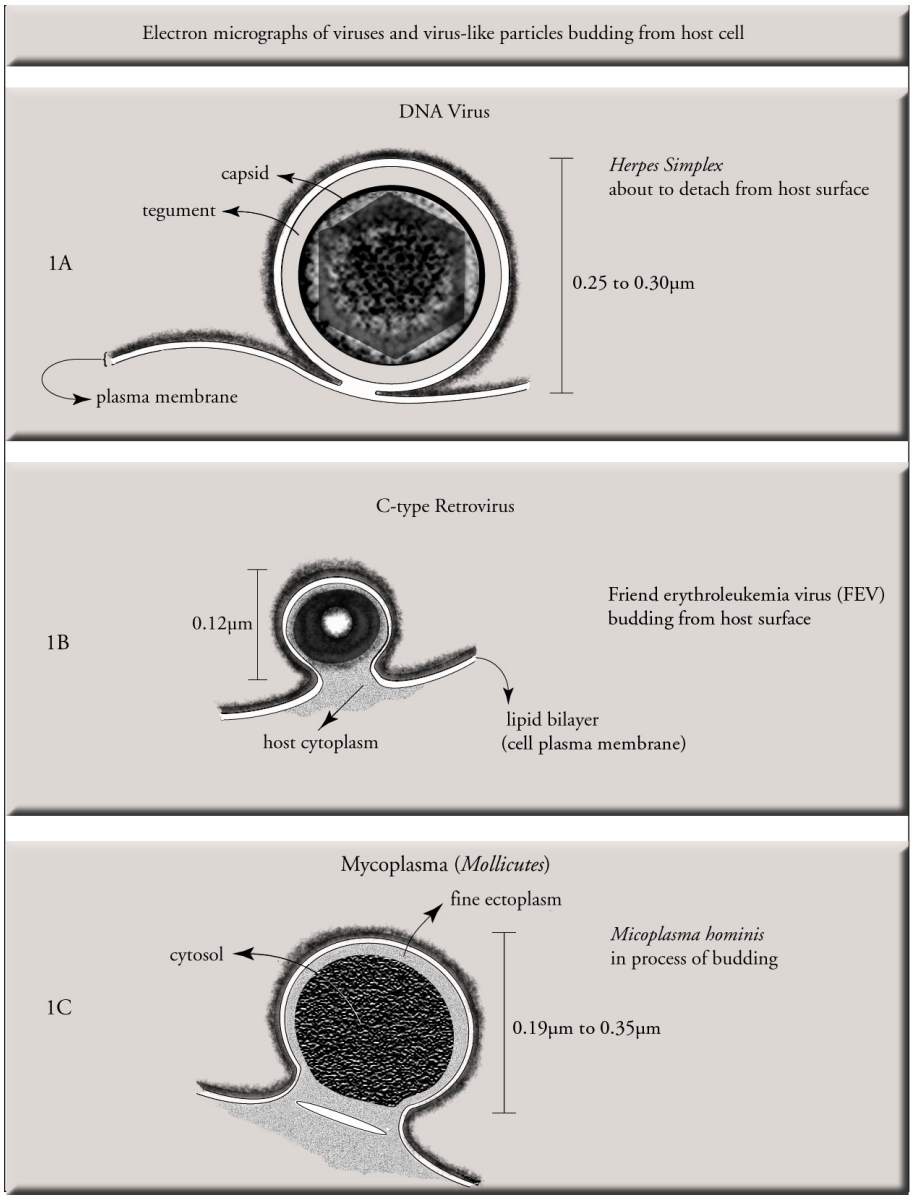

Fig. 1 - Representation of electron micrographs of typical DNA and RNA tumor viruses, and virus-like mycoplasma particles that are bacterial, budding from a host cell. The different particles can be readily distinguished under the transmitted electron microscope, but are ordinarily indistinguishable under light microscopy. In live mounts, however, mycoplasma have characteristic forms of motion (gliding, jumping). In fixed mounts, mycoplasma also stain Gram negative. Note that mycoplasma may be as small as 100 nm in diameter or slightly less.

Fig. 1A - *Herpes simplex 1* about to detach from a human host cell.

Fig. 1B- Friend erythroleukemia virus budding from murine host cell surface (osmium tetroxide stain).

Fig. 1C - *Mycoplasma hominis*, a virus-like particle, in the process of budding from a human host cell (after Barile 1967, Figure 2).

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ing an etiological relationship, as it turned out), and it was now possible to determine whether viral genomes were composed of DNA or RNA. The infectivity of purified RNA from Tobacco Mosaic Virus was demonstrated in 1956 [12].

In 1952, Renato Dulbecco devised the first *in vitro* assay for tumor viruses, employing tissue-culture of fibroblasts grown in monolayers [13]. Cells that became malignantly transformed by virus infection lost contact-inhibition, ceased producing a monolayer and adhering to the bottom of the culture vessel, to form distinct foci or plaques that, if harvested and inoculated into experimental animals, consistently produced the same fibrosarcomas from which the viruses had been isolated. It was now possible to produce and quantitate viral particles *in vitro*.

We should note that by the 1950's, it had also become well understood that chemical mutagens and ionizing radiation (from cosmic, solar, geological and man-made sources, including far ultraviolet light) could induce nonhereditary cancer, but the suspicion then arose that they might do so indirectly, via the production of viruses. During the 1920's and 1930's, most oncology research had gone into establishing that physical (X-rays) and chemical carcinogens acted as mutagens. The first two classes of identified chemical carcinogens were the nitrogen mustards and the nitrosoamines. By the 1950's, it became apparent that chemical mutagens (aromatic amines, polycyclic hydrocarbons, steroid hormones, asbestos) induced mostly epithelioid tumors, called carcinomas, whereas ionizing radiation seemed to induce melanomas, but most frequently leukemias.

Most generally, when viruses infect *permissive* cells two main responses occur. The acute infectious response is associated with massive viral replication and the lysis of the host cell, and is thus referred to as the *lytic or cytopathic* response. But persistent infection may instead take place, with the virus *either* (1) replicating independently of host replication (whether inserted into the host genome or leading an extra-chromosomal existence, eg an episome), and most often so slowly that it does not lyse the host cell; *or*, if unable to replicate, (2) with the virus being inserted into the host genome at or near critical gene sites, and leading a latent existence there (in DNA viruses and bacteriophages this is referred to as lysogeny proper; note that upon induction, lysogenic phages leave the chromosomal integration site and begin a lytic reproductive cycle). In the first case, one speaks of *chronic infection* (for example, infection of the trigeminal ganglion cells with the most common type of human herpes virus, HSV-I), and in the second, of a *latent infection* with no virus production.

2. DNA tumor viruses

It was early research with bacteriophages in the 1940's and early 1950's - on their ability to 'transform' bacteria - that led to interest in eukaryotic viruses and investigation of the possibility that viral infection might cause malignant transformation of the eukaryotic cells of metazoa.

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Early work with the murine polyoma and the African green monkey SV40 DNA viruses showed that, though *not* oncogenic in their hosts of origin, they could cause a variety of tumors when injected into newborn animals of susceptible hosts [14]. Infection of living animals or tissue culture cells exhibited alternative courses - a lytic and a transforming responses. Whereas the *lytic response* produced large numbers of viral particles in permissive cells, in a small number of nonpermissive cells that were transformation-susceptible the virus integrated in the host genome and yet no trace of it could be found (cell lines may be permissive to one DNA virus and nonpermissive to another; eg NIH3T3 cells are permissive for polyoma and nonpermissive for SV40). Infection with a single viral particle sufficed for transformation. Transformed cells presented no production of viral particles at all, these viruses behaving in these cells like the lysogenic phage of bacteria. However, it was remarkable that while integration of the viruses was observed in their hosts of origin, no transformation or tumor formation occurred. By itself, integration was not sufficient to initiate transformation [15], the latter requiring expression of virus-specific transforming antigens.

Human papilloma viruses are responsible for a variety of warts - benign epidermal tumors - but, according to zur Hausen's 1977 suggestion, these warts may convert into squamous cell carcinomas if exposed to X-rays or if they persist longer than 5 years [16]. Since then, DNA of cervical, uterine, vulvar and penile carcinomas was shown to contain human papilloma virus (HPV) homologous sequences in 61% of German patients - but only in 35% of Brazilian and Kenyan patients [17], which suggests the HPV presence could just as well be a coincidence. The viral homologous sequences are required for carcinogenesis and tumorigenicity [18], yet no intact HPV particles have been isolated from these carcinomas. Based on epidemiological considerations alone, these carcinomas appear to have a viral etiology of an infectious nature [18]. However, it is far from clear whether infection with HPV causes these various carcinomas, whether it requires super-infection with other viruses (such as HSV-2), or follows in the footsteps, or still whether it interacts with other systemic risks of neoplasia. As in infection with the Epstein-Barr virus (see below), these tumors cannot be the direct result of primary infection without some other factor(s) intervening, likely a multiplicity of factors. This is evident from epidemiological studies, as they show that only a very small fraction of those infected with HPV develop carcinomas.

Transforming abilities have been found for many DNA viruses, whether they integrated in host chromosomes (like polyoma virus, adenovirus, papilloma) or not (like fibrosarcoma-inducing pox virus). However, unlike oncogenic retroviruses (see below), DNA tumor viruses do not transduce cellular oncogenes; they transform the host cells mostly by the production of virus-specific proteins (eg the nuclear "T"-antigen characteristic of early infection with polyoma), or by targeted mutagenesis (insertions, deletions, inversions) that activates DNA transcription. The main trait of most DNA

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tumor viruses is that they appear to integrate in the host genome only randomly. There are two cautions to this: (1) that these studies have all been carried out with either laboratory animals or cell lines that were experimentally infected (and typically with high multiplicities of infection); and (2) that the translocations induced by the Epstein-Barr virus (EBV) appear to be selective.

Though the existence of animal models demonstrating causation of sarcomatous tumors by DNA viruses emboldened the belief that cancer had a viral etiology, the evidence for DNA viruses causing human cancers is, to this day, practically nil. In defiance of Koch's postulates, the epidemiology of cancer does not match the spread of any infectious DNA viruses, with the notable exception of the EBV. Even when DNA viruses are able to transform cells in tissue culture, like the herpes simplex viruses HSV-1 and HSV-2 that have high homology with EBV, none of the resulting transformants contain HSV [19], and there are no human tumors that can be causatively linked to them.

The herpesvirus family (*Herpesviridae*) was the main DNA virus candidate to play a central role in viral oncogenesis in humans. Some of the herpesviruses are present in virtually all human populations, and they are expressed in many human ailments and diseases - cold sores, shingles, otitis (and likely in the etiology of Ménière's Syndrome), venereal disease, infectious mononucleosis (once thought to be an infectious form of leukemia), birth defects, in the induction of Burkitt's B-cell lymphoma and, possibly, of nasopharyngeal carcinoma. They also account for many animal diseases. Herpesvirus B *simiae*, which only causes cold sores in monkeys, is the cause of a fulminating fatal encephalomyelitis in humans.

Herpesviruses are replicated inside the host cell, and their particles bud from the cell surface in a manner analogous to retroviruses (see **Fig1A** and compare with **Fig 1B**). As a consequence, they can be mistaken for the latter under high-power darkfield light microscopy and very low power electron microscopy, as well as for cell-adsorbed mycoplasma, all the more easily as these particles (viral and virus-like) have the same size range. The herpes virion (or nucleocapsid) is composed of a core containing an histone-packed, double-strand of two 10^5 kD linear DNA molecules, and a protein capsid. Replication of viral DNA takes place inside the nucleus of infected cells, and it typically (or most frequently) involves episomal circularization of the linear genome (analogous to phage DNA processing). The DNA polymerase produces multiple concatemeric DNA molecules to be packed into an equal number of virions. Inside the host cell cytoplasm, only nucleocapsid forms exist. The complete viral particle is present only outside of cells, whether in the process of being 'emitted' (released) from infected cells, in free state, or adsorbing to the surface of cells that it will subsequently infect. Outside the cell, the virion is frequently surrounded by an amorphous gel - the tegument - that it took from the host cytoplasm, and by a lipid bilayer envelope that it took from the host cell membrane during budding (see **Fig 1A**). Whereas the complete virus - the nucleocapsid - typically

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measures 180 nm in diameter (as is the case with the EBV), the complete virion typically measures 0.2 to 0.3 μm, so it is a 'large virus'.

Dennis Burkitt first observed that, given association with the spread of malaria, the geographical distribution of a B-cell lymphoma affecting East African children also appeared to be related to climactic factors [21]. In 1964, M. Epstein identified a herpesvirus in tissue cultures of lymphoblasts from a Burkitt's lymphoma cell line [22]. Only some of the lymphoblasts produced virus (which was cytopathic), and virus-producing cells could be increased to 20% with L-arginine depletion [23]. The acute form of infection with EBV is the now prevalent infectious mononucleosis (IM), a glandular fever which was once disseminated mostly among lower socioeconomic strata [24]. IM is a polyclonal lymphoid leukocytosis easily diagnosed by the vacuolated ("moth-eaten" appearance) cytoplasm of a significant portion (up to 35%) of the lymphocytes in peripheral blood (see **Fig. 2**). IM is an insidious disease that persists lifelong in peripheral blood B-lymphocytes, and may have episodic manifestations.

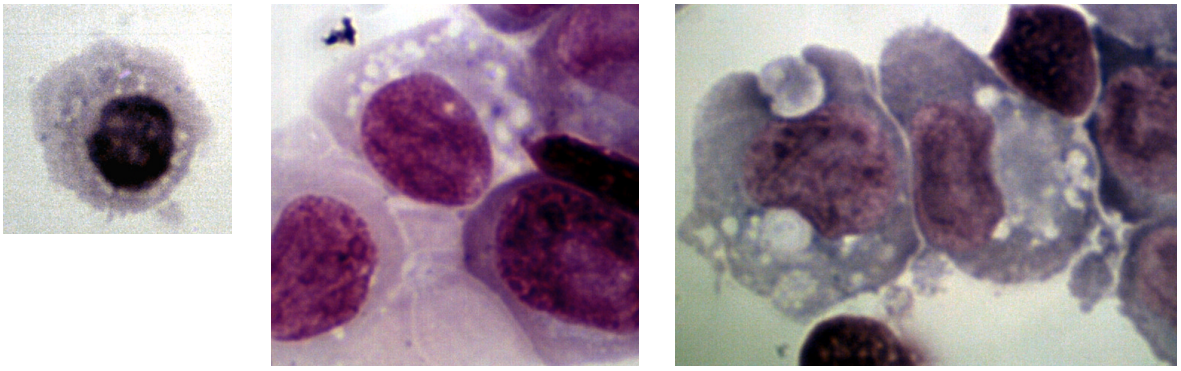

Fig. 2 - Starry-sky pattern of vacuolated CD19+ cells is characteristic of pre-pre B-cells ("large lymphocytes") in the bone marrow of patients with Burkitt's lymphoma, leukemia and other B-cell ALL. These abnormal lymphocytes are also present in peripheral blood (on the left, 1150x mag.), where they are easily mistaken for monocytes, and in cell lines derived from these leukemic patients (on the center and right, 1750x mag.). Luminera Infinity-1 camera, Wright's stain.

The absence of replicating EBV inside most Burkitt cells suggested the virus existed in some proviral form, and this was subsequently confirmed when it was found that all Burkitt cells carried multiple copies of the viral genome [25], most in the form of unintegrated circular DNA and a few, in some cases, integrated in the linear virion form [26].

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Originally it was thought that EBV infection and induction of transformation was restricted to B-lymphocytes, but its role in inducing nasopharyngeal carcinoma [27] in epithelial cells has now been demonstrated as involving linear virion integration in the genome of these cells [28-29]. EBV infection is thought to be predominantly carried out via the nasopharyngeal route [30].

The most distinctive *in vitro* trait of EBV infection of B-cells is the establishment of continuous cell cultures, ie *immortalization*. The EBV encodes a family of six nuclear antigens (EBNAs), one of which, EBNA-2, is the transcriptional activator [31] responsible for the induction of the B-cell activation antigen, CD23 [32], and for the immortalization of cells infected *in vitro* by EBV [33]. Thus, current thought is still that IM predisposes an infected individual to later on express a B-cell type of malignancy. Detection of anti-EBV antibodies indicates previous IM of varying severity, most primary infections being so mild that they went by unnoticed [34].

B-cells from patients with Burkitt's lymphoma present two main translocations that they share in common with other B-cell lymphomas (and thus are *not specific* to EBV-induced lymphomas): reciprocal 8 to 14 chromosome translocation [35] and a translocation of chromosome 8 to either chromosome 2 (t(2;8)) or 22 (t(8;22)) [36] (see below and **Table 2**). Both translocations involve activation of the immunoglobulin heavy chain gene IgH and the c-*myc* oncogene [37-38].

3. Retroviruses and their cellular origin: viral and cellular oncogenes

All RNA viruses capable of inducing tumors in animals happen to be retroviruses. The name 'retrovirus' comes from the enzyme REverse TRAnscriptase (RT), to give "retra". Retroviruses carry the *pol* gene that encodes for this enzyme, and they have the particularity of being the only RNA viruses with a diploid genome. In infection, retroviral particles in the range of 0.25 to 0.35 μm are adsorbed to host cell surfaces where they bind stereospecific glycoproteins of both cellular and viral origin (thus, while encounters with host cells may be treated as a chance occurrence, infectivity is not random but relatively specific), and subsequently penetrate through the plasma membrane (mimicking endocytosis). All retroviruses replicate by insertion of a double-stranded DNA copy (a *provirus*) of their RNA genomes into the genome of a host cell. A single-stranded DNA sequence (rDNA) is first produced from one of the RNA strands by the RT enzyme, and then a complementary DNA (cDNA) strand is polymerized. A major step in the understanding of the function and origins of retroviruses was the independent discovery of reverse transcriptase in 1970 by Harold Temin's group [39] and David Baltimore [40], which confirmed Temin's 1962 hypothesis that RNA oncogenic viruses reproduced in the form of a DNA provirus copy that integrated in the host genome. Identified in oncogenic retroviruses, RT synthesized rDNA, ie complementary DNA from an RNA template - breaking the first rung of the so-called DNA dogma: genetic information also flowed in reverse from

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RNA to DNA. Temin went as far as suggesting that retroviruses functioned as intercellular messengers with a role in differentiation and "could provide part of a mechanism for inheritance of some acquired characters".

Integration into the host genome is part of the lifecycle of retroviruses, though proviruses may exist in single, circular episomal form, typically prior to integration. Dynamic states of equilibrium between integrated and episomal proviruses may be a strategy of the virus to retain a potential for multivariant adaptive change [41]. Similarly, it may also be a strategy of the host cell itself. Once integrated, replication-competent retroviruses can generate either ordinary m-RNA transcripts or genomic RNA transcripts, each 6 to 10 kbp long. The latter are then joined to form double stranded RNA and packed within a virion (a protein capsule), with virion proteins having been translated from the viral m-RNA transcripts. No other known viruses are packed in double-stranded form inside virions (see **Fig.s 1B & 3**). Replication-competent retroviruses are also the only known viruses that can transform host cells while simultaneously replicating inside of them, and do so independently of synchronism with cell replication (note that they would thus differ in this from the classic scheme of organelle replication).

The replicated retroviral virion buds from the host cell surface by taking with it a bit of its cortical or gelated cytoplasm (see **Fig. 1B**), along with a portion of the plasma membrane (note that this parasitic graft is a residual marker of the truly *heterogenic ontogeny* of retroviruses). At high magnification with special techniques of light microscopy, budding of retroviral particles from a host cell is indistinguishable from the budding replication of mycoplasma from filaments of a parent mycoplasma that adsorbed to the surface of host cells (see **Fig. 1C**). Both would appear as if heterogenically created from the host, yet neither one likely is - the retrovirus being a replica of an infecting particle absorbed by the host cell sometime in the past, and the mycoplasma dividing by budding from another mycoplasma or its common stalk frequently embedded in the plasma-membrane of a previously infected host cell. Today there is, of course, a greater acceptance of the notion that at least each type of retrovirus must have *once* arisen *heterogenically* from cells (see below), *from cellular genes*, but this notion only applies to singular events of speciation (whether successful or not), and *not* to the ordinary host-dependent - but non-synchronous - replication and packaging of retroviruses in infected cells, which is the routine case when one observes the budding of a retroviral particle from a host cell surface with light or electron microscopy.

In a manner of speaking, the speciation of every viral type could be said to be 'endogenous', and precisely to the extent that viral ontogeny must be heterogenic. 'Endogenous' would here be opposed to what is 'infectious' or gained by infection, but would be so by subtending the notion of heterogenic ontogeny [42]. But modern molecular biology of retroviruses conceptualizes 'endogenous

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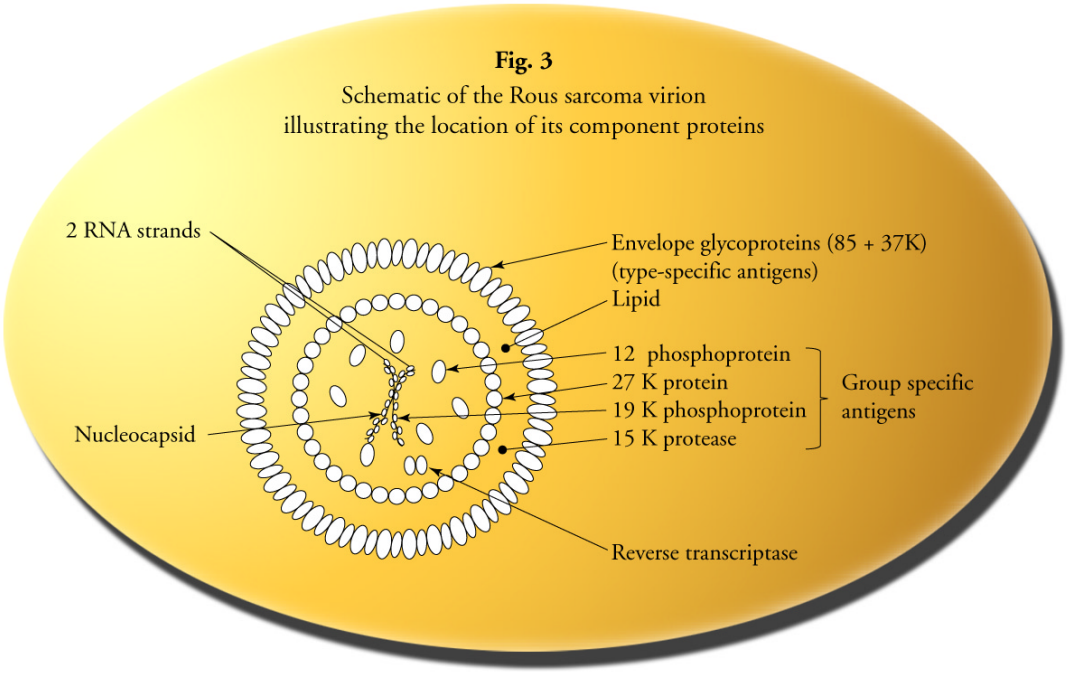

Fig. 3
Schematic of the Rous sarcoma virion
illustrating the location of its component proteins

viruses' rather differently - even though also in opposition to the concept of 'viruses acquired by infection'. Molecular genetics defines as 'endogenous' all viruses that are transmitted genetically to offspring and maintained as normal Mendelian genes. Vertical transmission (as opposed to horizontal transmission by infection) depends upon integration of viral DNA into the genome of host germ cells. Some endogenous viruses are silent (latent, ie under repressor control), others partially expressed and still others fully expressed and replicated. In evolutionary terms, the consensus is that retroviruses are recent "acquisitions". But this does not explain what their source is, whether they first infected animal cells as if coming out of nowhere, or were first created and emitted by animal cells themselves. Gross held that integration and vertical transmission were second to horizontal acquisition [43]. This is likely the case in laboratory investigations that use high multiplicities of infection. However, after the discovery that information exchange flows from DNA to RNA but also in reverse, back to DNA, Temin's "protovirus hypothesis" [44] explicitly considered that mutations and unusual recombinational events might create tumor viruses *de novo*, ie *heterogenically* [45]. This view was practically a neo-Lamarckian perspective on the creation and evolution of retroviruses that challenged the more traditional viral oncogene view of retroviruses which pins these down to either vertical transmission or horizontal infection. By admitting to the *de novo* creation of viruses from reshuffled genomic sequences carrying the requisite genetic elements, or from rescued viral footprints for that matter, the protovirus hypothesis questioned whether retroviruses and their involvement in specific oncogenic processes

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really functioned in the traditional sense of Koch's postulates as agents or causes of disease, and invited speculation as to whether viruses were mere symptoms of the disease - messages emitted by cells subject to degenerative or pathogenic processes, messages that targeted and altered hormonal (growth factor) networks that controlled and modulated cell metabolism, growth and differentiation. There were sets of cellular genes that had a capacity to escape the genome by utilizing viral packaging, and tumor formation might simply reflect errors in the back and forth flux of genetic information that affected the 'escapist genes' and might prompt their escape.

The central notion of the protoviral theory of oncogenesis is that retroviruses transduced cancer-causing genes (oncogenes) that were originally derived from host cell DNA (from "proto-oncogenes"), but which were no longer tissue dependent for their regulation and expression. This evolutionary relationship was evident early on when high homologies were found between the viral oncogenes and the host genomic sequences of the proto-oncogenes, indicating that these oncogene sequences were highly conserved between cells and retroviruses. Whereas proto-oncogenes encoded proteins or enzymes - typically phosphorylating kinases (tyrosine kinases, but also threonine and serine kinases) - that regulated normal cell growth and development, and whereas their expression was both tissue-specific and developmental stage-specific, viral oncogenes encoded mutant variations of these gene-products. In particular in retroviruses, these oncogenes had lost intron and regulatory sequences present in proto-oncogenes, this fact pointing to their heterogenic origin from cellular mRNA rather than cellular DNA. Expression of retroviral oncogenes is solely regulated by long, repeating nucleic acid sequences present at the start and end of their genome, and called long-tandem repeats (LTRs). A typical retroviral genome structure is shown in the upper part of **Fig. 4**, for the Moloney Murine Leukemia Virus (Mo-MuLV).

The facts that there are hotspots for the genomic integration of retroviruses, and that these are preferentially located near or in the sequences of cellular proto-oncogenes also pointed to the cellular origin of retroviral oncogenes. The marked cellular tropism of a retrovirus is a function of the oncogene it transduces. So it might not be surprising if the targets of retroviruses turn out to be growth factor receptors that control cellular growth, differentiation and metabolism.

In normal cells, there are no integrated defective retroviruses with their viral oncogenes (v-oncs), only active cellular oncogenes (c-oncs) under normal regulation and the odd normal retrovirus without a v-onc. And, indeed, all viruses found in normal or wildtype germ cells do not seem to carry a v-onc. This, as Arthur Axelrad taught these authors, led MacFarlane Burnet once to state that since the syringe of the investigator was the only vector of viral cancer, viruses could not be taken seriously as causative agents in human cancer and the successful induction and transmission of cancer by viruses in animals were mere laboratory curiosities without clinical value.

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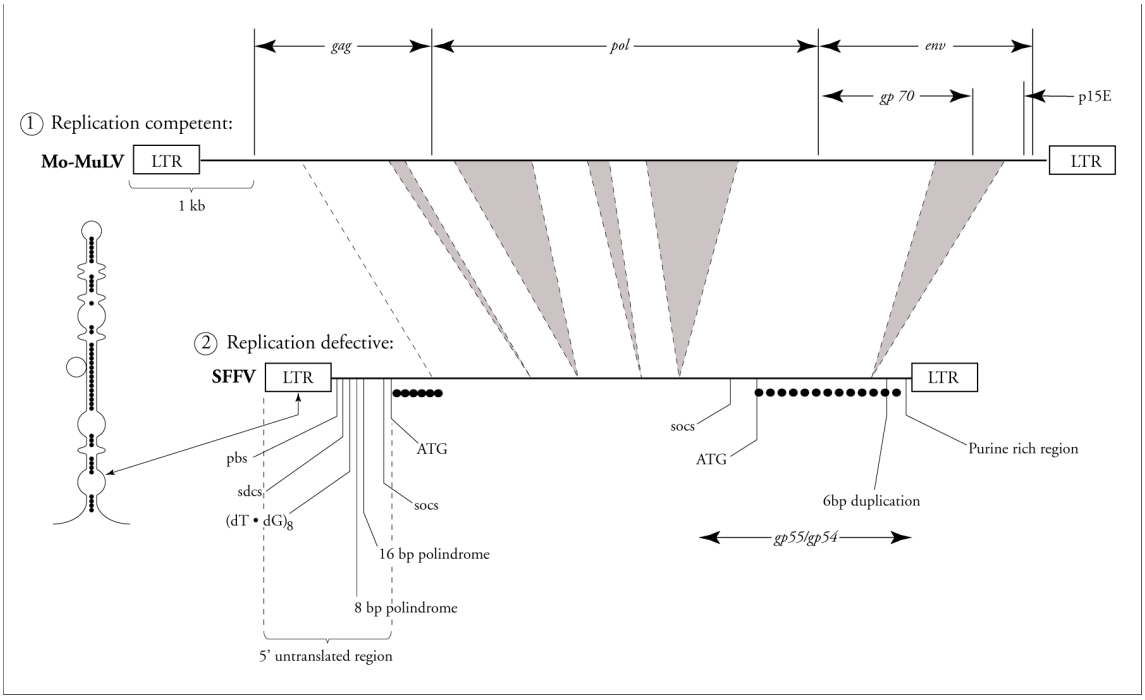

Fig. 4 - Typical genomic structures of replication-competent (Moloney Murine Leukemia Virus, Mo-MuLV) and replication-deficient (Friend Spleen Focus Forming Virus, F-SFFV) retroviruses. The latter have large deletions in the *pol* and *env* genes. The Friend SFFV comes in two varieties, anemia-inducing and polycythemia-inducing. The latter produces a surface protein (gp55) from the *env* gene that renders host cells (erythroid progenitors) independent of EPO, the hormone that normally regulates RBC production.

In evolutionary terms, the biopoiesis of retroviruses is indistinguishable from their heterogenesis from host cells: retroviruses and their oncogenes evolutionarily arose from cells, and not the other way around - that cells acquired oncogenes because they were infected with retroviruses. Since, ontogenetically speaking, infection must have come second with respect to true endogenous virus emission or production, viruses should be viewed as *cellular signals*. The fact that, whether by the adaptive pressure of the syringe or by nature, they can be induced to pick up cellular oncogenes and transduce them in a mutagenized form, or affect expression of critical cellular genes by nonrandom hotspot insertion near them, demonstrates that they are cellular signals *designed* to alter the normal growth, metabolism and differentiation of tissue. Their vertical transmission identifies simply an hereditary predisposition to possible neoplasia, just as their lateral transmission defines infection,

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which depends not just upon their infectivity potential, but also on encounters with hosts that are susceptible, genetically and dynamically (see below).

4. The broad classes of retroviruses

Two broad classes of retroviruses initially appeared to exist: leukemia-inducing and sarcoma-inducing. Retroviruses that transform fibroblasts in culture only induce formation of solid tumors (sarcomas) in connective tissue, whereas most retroviruses only induce hyperplasia (not neoplasia) of blood cells, and are thus known as leukemia (leukocytosis-inducing, to be more exact) viruses. The *transforming* sarcoma-inducing retroviruses are typically replication-defective (mostly due to lack of a complete viral envelope-coding *env* gene, see **Fig. 4**); once inserted into the host genome, they replicate only when the host genome replicates. They become therefore latent viruses. Whereas the leukemia-inducing *nontransforming* retroviruses were considered to be replication-competent but not cytopathic, ie they did not lyse the host where they independently replicated (see **Fig.s 4 & 5**). Sarcoma-inducing retroviruses can only replicate independently of host cell replication when rescued by a leukemia-inducing retrovirus that is referred to as a "helper" virus.

For a long time it was thought that there were no carcinoma-inducing retroviruses, but that retroviruses or their genes would mediate, as co-carcinogens, the effect of chemical mutagens in carcinogenesis. Nontransformed fibroblast cell lines that were "*spontaneously* immortalized" in culture (it is unknown how) could be transformed into tumor cells by chemical mutagenesis; moreover, whereas primary rodent fibroblasts failed to transform when exposed to certain chemical mutagens, exposure after infection with murine leukemia viruses resulted in transformation [46]. A two-step model of induced carcinogenesis was proposed, where viral infection worked as an 'initiator' that altered a normal cell into a pre-malignant cell, and the chemical or physical mutagen functioned as a 'promoter' inducing neoplastic transformation. The infecting virus might be defective and remain silently integrated until the mutagen intervened to induce its expression or activity and result in neoplasia. We should remark that this was the beginning of a new conceptualization of cancer as a process that crossed defined stages with distinct characteristics, initiation events being required before fully developed neoplasia manifests itself. Oncogenesis could therefore be thought as a vector of transformation, with pre-neoplastic lesions launching the vector. Thus the concept of an oncogenic vector emerged directly from the study of viral oncogenesis.

The broad distinction of two classes of retroviruses was subsequently corrected with the discovery of a carcinoma-inducing retrovirus with strong oncogenicity [47], that contained two unrelated and independently expressed oncogenes, v-*myc* and v-*mil(raf)*. Localization of the corresponding proto-oncogenes which the virus transduced also showed these were unlinked [48]. Transformation by

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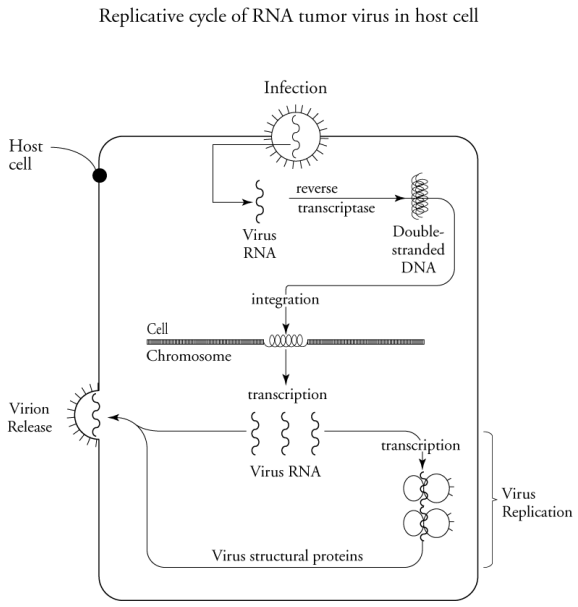

Fig. 5 - Diagram of the reproductive cycle of replication-competent retrovirus, from infection (at the top) to extrusion of a complete replicated viral particle (at the left) budding from the host cell membrane. Hundreds of copies may be produced by the same cell.

Myc/Mil(Raf) proteins was a cooperative event that - by increasing the expression of c-Fos and c-Jun proteins (more on this below) - activated DNA transcription of genes implicated in the control of cell migration, invasion and metastasis [49].

5. Retrovirus-induced immortalization, transformation and the differentiation switch

5.1. Immortality and transformation

Central to the determination of whether leukemia-inducing retroviruses were able to induce neoplastic transformation was the distinction between induction of *immortality* and *malignant transformation*, which required determination of what exactly were the obligatory and universal traits of the latter and the conditions under which preneoplastic cells progressed to full neoplasia. The relation between immortalization and transformation was from the beginning inseparable from the conceptualization of a 'tumorigenic process' (the 'oncogenic vector') that crossed distinct stages and, through successive epigenetic and then genomic and phenotypic alterations, finally produced fully tumorigenic, malignantly transformed cells. Thus oncology would come to realize that immortalized cells were not transformed neoplastically, and not all malignantly transformed cells were tumorigenic or had the same tumorigenic potential.

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Normal diploid mammalian cells have a limited lifespan and proliferative potential, clocked by the number of their cellular divisions, with a final phase of senescence before they die (see **Fig. 6**). This sequence of events is observed regularly in tissue culture of primary cells. In contrast, immortalized cells can give rise to cell lines (even though the precise origin of these adaptations to tissue culture has not, for the most part, been to this day established) because their proliferative potential is unlimited (see **Fig. 6**). Chemical mutagens can induce immortalization [50] (even though these tests were performed with sera that, back when these studies were conducted, were still commonly contaminated with mycoplasmas, not to mention chemical contaminants that are part of serum, such as undefined toxins, including bacterial endotoxin, and various growth and regulatory factors). So can infection with certain viruses.

Most importantly, all malignantly transformed cells have been immortalized, but not all immortalized cells are malignantly transformed - ie capable of tumor formation or tissue invasion - or present gross genotypic alterations characteristic of neoplastic cells. Immortality is an essential step in the process of the malignant transformation of cells [50-51]. Defined DNA fragments from *Herpes simplex* virus type 2 (HSV-2) were able to either immortalize *and* transform, *or just* immortalize, embryonic rodent cells grown *in vitro*, again indicating that neoplastic transformation involved at least two steps [50]. Experiments like these led to the hope that there might be universal pathways to oncogenesis, that only a few oncogenes might be involved, and that, at least with respect to the transformation of the immortalized stage, single-hit event kinetics might suffice [52]. Yet, HSV-2 is not tumorigenic *in vivo*. Nevertheless, the *in vitro* findings appeared to tally with the oldest phenotypic model of oncogenesis, which held that cells first reversed or regressed to an embryonal stage (*anaplasia*), recovering some pluripotential state and engaging in some degree of excessive proliferation (*hyperplasia*), and only afterward would they become malignantly transformed into some other tissue (*metaplasia*), thought of *either as an amoeboid 'tissue'* (a tumor can be compared to a colony or colonies of protozoa, including parallel tissue-invading abilities) *or a malignant and abortive variant of embryonic differentiation*. The malignant transformation of the cell was '*metaplastic*' and the tumor-seeding ability was '*neoplastic*' (growth of 'new tissue').

In this model, immortalization or non-senescence would likely be connected to pre-neoplastic, hyperplastic stages of proliferation. However, hopes that either immortalization or transformation could be achieved by simple event kinetics would evaporate by the late 1990's, as it became clear that cell lines were established by virtue of mostly undefined changes in large arrays of very different types of genes, and not just changes in oncogenes and tumor-suppressor genes. Even induction of immortality is not an all-or-none process, presenting "different degrees of reduction in the commitment of cells to a non-proliferative state" [50]. Likewise, there are different degrees of transformation.

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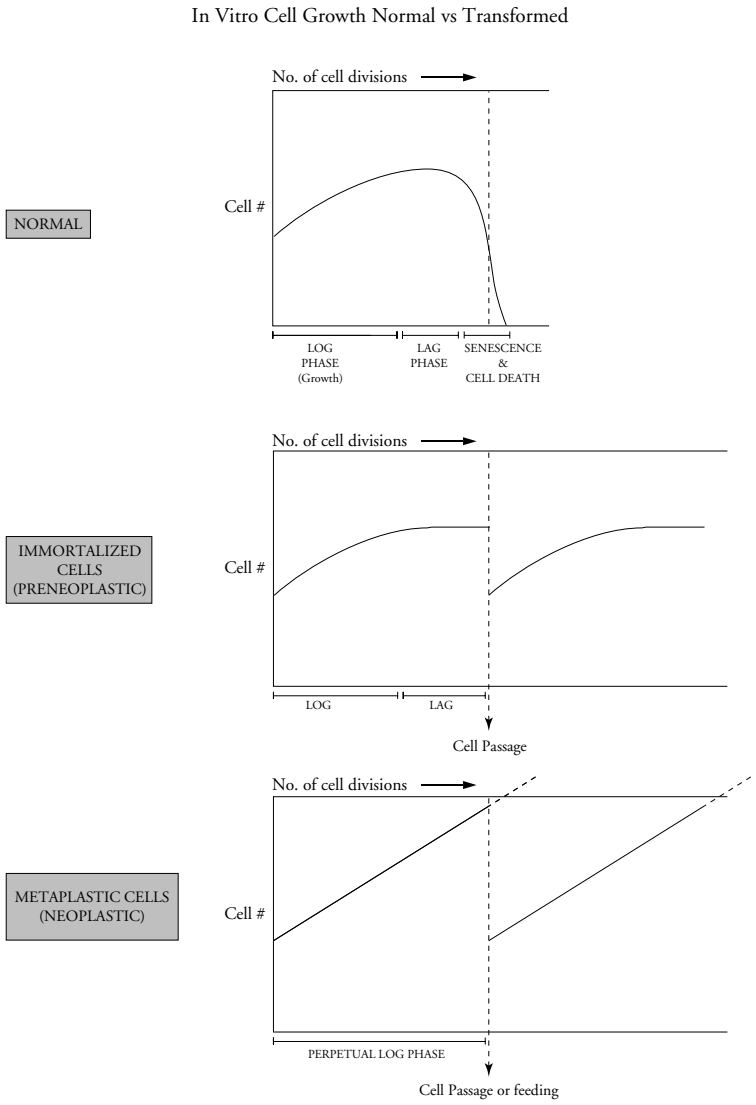

Fig. 6 - Typical *in vitro* cell growth curves for normal primary cells (top), immortalized cell lines (middle) and malignantly transformed cells (bottom).

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5.2. What are the minimal characteristics of malignant transformation?

The main problem that these realizations brought to light was the necessity of agreeing on the minimal criteria of malignant transformation. Neoplasia is manifested by the abilities to alter the normal order and morphology of tissue, to invade normal tissue and grow tumors (tumorigenicity). Today, it is generally assessed *in vivo* by tumorigenic assays in nude mice (hairless mice that lack T-lymphocytes since they have no thymus), given that fibroblast *in vitro* focus-forming assays cannot reliably assess tumorigenicity (all the more so as they are most frequently contaminated with serum).

What do these tests and assays tell us about the minimal internal changes, genotypic, metabolic and phenotypic, that a cell must undergo in order to become malignantly transformed?

In primary fibroblast cultures, it was evident what were the signs of transformation: *loss of contact inhibition* that suppresses both cellular motion and DNA replication; *very fast rates of growth and replication* at higher cellular densities; loss of adherence capability or *acquisition of anchorage independence* (tested by growth in agar or liquid suspension); acquisition of *spindle cell forms* (change from fibroblast to epithelioid phenotype), and acquisition of *some degree of motility* correlating with the loss of actin cables. These were histological and biochemical alterations characteristic of an '*amoeboid-becoming*' on the part of fibroblasts infected with an oncogenic virus - to which was added their ability to reproduce indefinitely and form recognizable and countable foci.

Thus, neoplastic cells were necessarily hyperplastic; they grew and divided at rates ca 20x faster than normal cells. Foci culture also afforded other markers of malignant transformation confirmed by the correlated sarcomagenic ability: whereas normal cells required high-serum in undefined cultures, the serum requirement of transformed cells was minimal; uptake of glucose increased and so did lactic acid production in cultures of transformed cells; the large external trypsin-sensitive (LETS) protein was absent; proteolytic enzymes were typically activated; surface antigens and receptors were altered; and agglutination of plant lectins was observed. The changes in glucose uptake and lactic acid production clearly identified an alteration in metabolism, and we will address this critical marker of malignant transformation in the next communication.

However, if the conjunction of these traits appears to be required by truly tumorigenic cells, not all cells presenting some of these traits are necessarily neoplastic or tumorigenic. This distinction finally became fully apparent when it was observed that different types of leukemia retroviruses induced distinct *in vitro* alterations of chicken fibroblasts: there were RSV-like transformed cells with microvilli, no actin cables, decreased LETS protein, fast division rates (hyperplasia) and increased glycolytic metabolism; and there were AEV(avian erythroblastosis virus)-like 'transformed' cells, with bleb-like protrusions, no actin cables, also a fast division rate (hyperplasia) but with normal LETS protein and no increases in glycolysis or proteolytic activity. Only the former were sarcomagenic [53].

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What about changes in genotype that are markers of transformation? Here, the diversity of genes hit by neoplasia made the task simply daunting (see below). There were no universal cancer genes, no genes universally altered when cells were malignantly transformed, and oncogene activation alone was not sufficient for neoplasia. But eventually it became apparent that specific neoplastic phenotypes were consistent in the varied sets of genomic alterations they presented. We shall return to this below and in the following papers.

Could one take viral integration in the host genome always as a necessary sign of transformation? Again, the answer to the last question turned out to be "no", since *most* human cancers, for example, do not exhibit either a latent viral infection or viral-induced transformation.

Modern oncology tends to describe the malignant state by a set of minimal criteria. We will try to systematize these, in the most consistent fashion we believe is presently possible:

1. A switch from aerobic (respiratory) to anaerobic metabolism, graduated *in vivo* by location of the neoplastic cell with respect to the decreasing gradient of oxygen concentration inside tumors (we will discuss this in detail in the next communication). All proliferating cells, and not just hyperplastic ones, rely on glycolysis, and the increased rates of glycolysis in cancer cells correlate with the increased rates of cell division (proliferation).

2. Acquisition of an immortalized state that suspends programmed senescence. Benign growths (*viz* virus-induced warts, etc) present hyperplastic states without neoplasia or ability to invade tissue, and, in some cases, they may be conceived as pre-neoplastic processes. The frequent evolution of bladder papillomas into carcinomas is an example of a typical process of conversion of hyperplastic, benignly transformed cells into neoplastic, malignantly transformed cells (see **Fig.** 7).

3. Non-responsiveness to control by normal growth and differentiation factors, generally translated either by some hypersensitive response to one or more of these factors, or by independence from them. This trait affects a multiplicity of intracellular kinases that regulate metabolism, growth and differentiation. In general, it encompasses two distinct modifications of cellular behavior (constitutive of the 'amoeboid becoming'):

3.1. Non-responsiveness to "Stop dividing" signals, which results in hyperplasia and the loss of contact and DNA-replication inhibitions.

3.2. Non-responsiveness to "differentiation-Go" signals.

It seems, however (see below, on Friend disease), that oncogenesis may, just as well, encompass abortive implementations of "differentiation-Go" signals, these being precisely what results in metaplastic phenotypes and, eventually, cell death.

4. An array of genomic alterations, some epigenetic, others adaptive, involving any of the following - gross chromosomal aberrations, chromosomal or DNA duplications, epigenetic alterations

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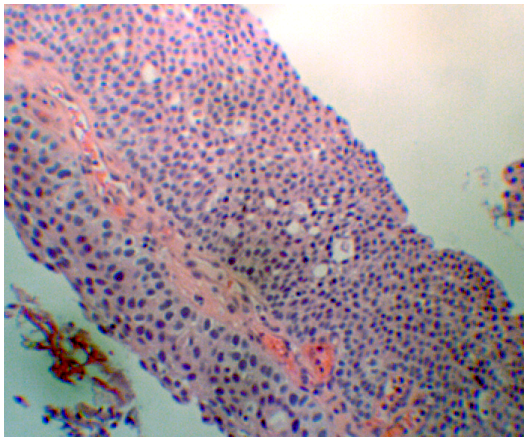

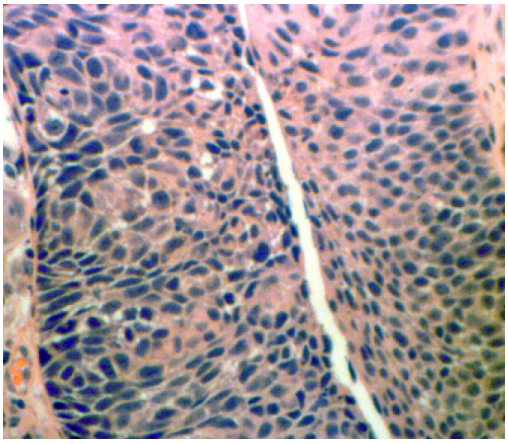

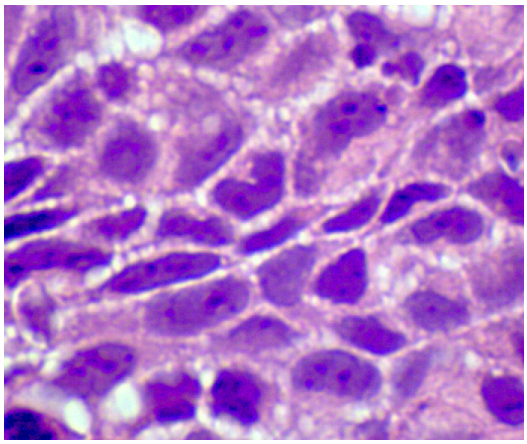

Fig. 7A (top left) - Contrast between normal transitional epithelium of the bladder (left side of plate) and nearly contiguous, hyperplastic epithelium of a papilloma (right side of plate). Luminera Infinity-1 camera, H & E stain. Mag.: ~50x.

Fig. 7B (top right) - Contrast between the hyperplastic benignly transformed epithelial cells of a human bladder papilloma (right side), and the apposing neoplastic cells (atypic and spindle) of a contiguous carcinoma (left side). Luminera Infinity-1 camera, H & E stain. Mag.: ~125x.

Fig. 7C (above, right) - High power view of atypic, spindle and high vacuolized neoplastic cells of the carcinoma shown in **Fig. 7B**. Luminera Infinity-1 camera, H & E stain. Mag.: ~800x.

in gene expression and mutagenized genomic sequences with altered gene sequences and altered gene transcription. Most importantly, the genomic alterations involving chromosomal aberrations or genetic mutations, though very diverse, tend to occur at insertional, deletional or breakage hotspots that affect cellular oncogenes and other genes involved in the regulatory control of cellular growth, metabolism and proliferation. Similarly, gene amplification by chromosomal duplications or other pathways typically involve the same classes of cellular genes.

5. The ability to invade tissue, form tumors and metastacize, and thus behave like foreign amoeboid cells. In general, tumorigenic cells are all the more aggressive as their metabolism becomes strictly glycolytic, their rates of proliferation become 'wild' and their genomic alterations profound and extreme.

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5.3. Transformation by acute leukemia-inducing retroviruses and the problem posed by differentiation of leukemia cells

Another key discovery was the realization that leukemia-inducing retroviruses could elicit malignant transformation of infected cells. All such retroviruses were - like the sarcoma-inducers - replication-defective and induced acute leukemias once integrated in the host genome. Cases in point were the latency-free murine Friend erythroleukemia virus(es) (isolated by Charlotte Friend in 1957 from the cell-free extracts of ascites tumor cells in newborn mice [54]), and the avian erythroblastosis virus (AEV) [55].

Initially, it was thought that replication defective retroviruses created latently infected cells devoid of virus production and were capable of inducing acute leukemia if they carried an oncogene that specifically affected cell metabolism and growth control, whereas replication-competent retroviruses that typically did not carry an oncogene created chronically infected cells and only slowly induced leukemia (typically by insertional mutagenesis, as in c-*myc* activation by avian leukosis virus, ALV, that causes chicken lymphomas). However, acute leukemias also appeared to be generated by replication-defective retroviruses that did not carry an oncogene, through modification of the viral envelope gene *env* (typically encoding for gp70), such as the gp55 mutation characteristic of the spleen focus-forming virus, SFFV (see **Fig. 4**) [56].

Moreover, the question remained whether these viruses induced a real transformation of the infected hosts. Friend virus (FEV) is a mixture of a helper (F-MuLV) and a replication-defective virus (SFFV). FEV, in its two variants, FV-P and FV-A, induces a rapid erythroleukemia (an erythroblastosis) accompanied by either anemia (lack of mature RBCs) or polycythemia (excess of RBCs), according to the SFFV strain (see **Fig. 4**) that it packs - the anemia-inducer (SFFV-A) or the polycythemia-inducer (SFFV-P) strains [57]. Whereas the anemia strain does not alter the normal sensitivity of erythroid precursors to the physiological hormone EPO that regulates erythropoiesis, infection with the polycythemia strain induces EPO-independent erythropoiesis [58-61]. Both strains are unable to transform fibroblasts in culture. Mice infected with FV-P become polycythemic within a week after inoculation [61] and FV-A infected mice became anemic within the same time period [54]. The late stage of Friend disease is characterized, in turn, by hepatomegaly [54, 62] and above all, by the frequent emergence of neoplastic cells transformed by the Friend virus [63-66]. In 1964, Axelrad and Steeves reported that intravenous injection of FV-P preparations produced macroscopic foci on the spleen surface of treated mice (see **Fig. 8**) [67]. The titer of SFFV particles could be quantitated as an existing linear relation between the mean number of spleen foci counted (at 9 days after infection) and the dose of FV-P given to susceptible mice (see **Fig. 9**) [67]. Using the spleen focus-forming assay, it was then determined that whereas FV-P strains yield high SFFV titers [58, 67], FV-A strains yield low

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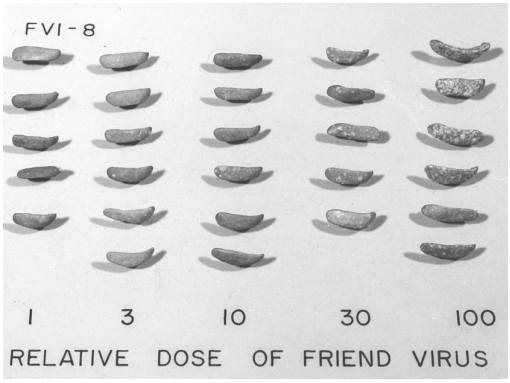

Fig. 8 - Spleen focus-formation 9 days after infection of susceptible mice with Friend virus, as a function of the viral dose administered. Doses are expressed as equivalent to the number of infected spleen cells from which the virus was extracted (After Axelrad 1966 reference [**100**]).

SFFV titers [**68**]. Mouse and rat clones, which were non-productively infected with SFFV-P, were used by Alan Bernstein's group to show that this defective virus could be rescued by superinfection with F-MuLV - this being assessed *in vivo* by the spleen focus assay [**69**]. These findings also indicated that F-MuLV was required for spleen focus formation, though not sufficient for it.

The first cell lines immortalized by the Friend virus were established by Friend et al from subcutaneous tumors passaged serially [**70**]. They have become known as Friend erythroleukemia cell (FLC) lines. Most FLC lines have been established from neoplastic growths that emerge in the late phase of Friend disease (after the 4th week following infection with FV-P and after the 8th with FV-A [**71**]), either from subcutaneous tumors or from the spleens of leukemic mice (see [**72**] for a review). These FLC lines are routinely passaged in serum-containing liquid culture. FLC lines have been noted to form an heterogeneous population composed of (1) sarcoma-like reticulum cells - which resemble the tumors of origin - and (2) cells at various stages of spontaneous erythroid differentiation [**71-72**]. Nevertheless, when FLC suspensions are injected subcutaneously, the tumors they yield lack any apparent erythropoiesis and only contain the first type of cell [**64, 71, 73**]. Whereas early FLC lines (dl5) proliferate rapidly but have a high rate of turnover and limited mitotic activity [**74**], late FLC lines have extensive proliferative capacity and a lesser probability either to die or to spontaneously differentiate [**72, 74-77**]. Late FLC lines have the particularity of being inducible by dimethylsulfoxide (DMSO), but not EPO, to undergo terminal erythroid differentiation [**76**] and hemoglobinization [**72, 77**]. SFFV-free F-MuLV-infected cell lines differ from FLC lines in colony morphology and in that neither DMSO nor EPO can induce them to hemoglobinize [**78**].

Demonstration of SFFV's ability to induce splenomegaly [**69**] cannot be expressed separately from the rescuing action of the helper virus, which contributes only functions necessary for the replication of SFFV (see **Fig. 4**). In fact, the F-MuLV helper has no spleen focus-forming ability, but it is

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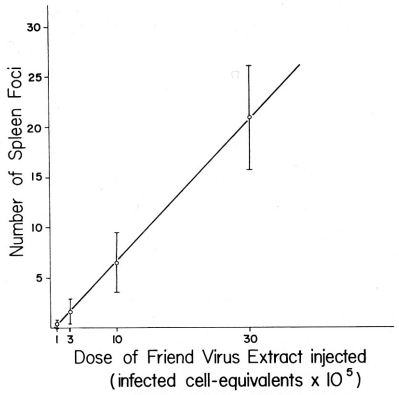

Fig. 9 - Number of spleen foci versus dose of Friend virus extract injected in susceptible mice (after Axelrad & Steeves 1964 reference [67]).

capable of *slowly* inducing erythroleukemia [79-80]. However, SFFV also appears to be sufficient for late stage erythroleukemic transformation, without helper virus [81]. Yet, such late transformed cell lines could still be induced to restrict their proliferation and differentiate. Nevertheless, tumorigenicity and capacity to metastacize increase as the transformed cells lose the ability to differentiate upon chemical induction [82]. Together with Axelrad, we confirmed the heterogeneity of FV-P-induced FLC lines and observed a shift of the modal volume with counter-current centrifugal elutriation - accompanied by decreased cell viability and increased cell death - 48h after DMSO induction (see **Fig. 10**) [83]. Whereas modal populations prior to induction had high rates of proliferation and low erythroid commitment, *modal populations after induction* contained mostly cells committed to terminal proliferation and erythroid differentiation. For the fraction obtained at the counter-current flow rate of the shifted modal population, the number of erythroid colonies (d7) and bursts (d15) doubled upon induction with DMSO (see **Fig. 11**). Yet, the shifted modal population showed no altered expression of their EPO receptors [83-84], once again indicating that the FLC differentiation was independent of EPO and not mediated by increased EPO receptor expression [83-88].

The heterogeneity of FLC indicated that one of the subpopulations cycled constantly, like a restricted stem cell (a progenitor cell) pool, whereas the other subpopulation resulted from an ongoing 'spontaneous' commitment to *simulated* erythroid differentiation, and it could be substantially increased at the cost of the first subpopulation by DMSO induction. The facts indicated that the differentiation of FLC was, in all cases (spontaneous or induced), abnormal. There was, nevertheless, a switch between the malignantly transformed state of the modal subpopulation of a Friend line and a state of commitment to undergo some form of erythroid differentiation. Remarkably, in some aspects, the EPO-independent differentiation of FLC evokes human, rather than murine, erythropoiesis. Attachment of Friend cells to glass or incubation with atmospheric oxygen for a few hours were

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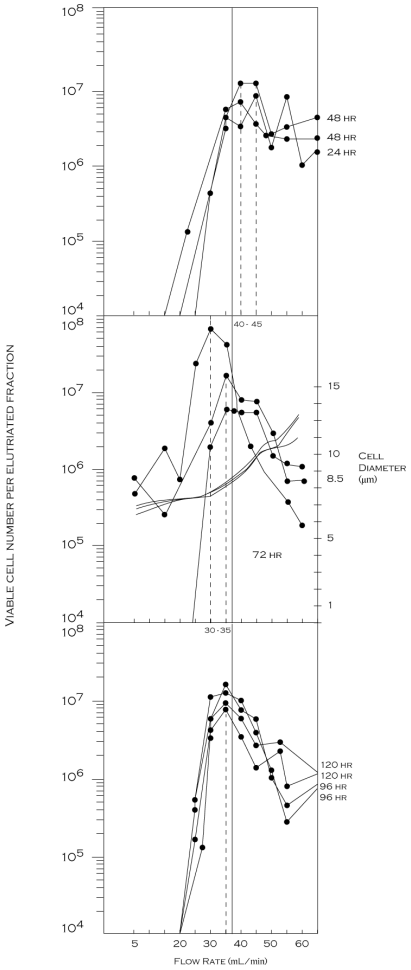

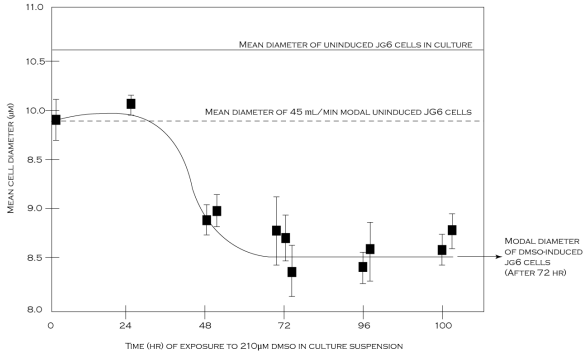

Fig. 10A (left) - Ten counter current centrifugal elutriations at 24 hr. intervals after induction of FV-P-infected JG6 cells with 210 mM DMSO. Middle panel also shows cell size curves (projected onto the right ordinate) for all 72 hr. elutriations. The elutriated modal subpopulation shifts from 40-45 mL/min prior to DMSO induction, to 30-35 mL/min at 48-72 hr. after induction.

Fig. 10B (top right) - Variation of mean cell diameter in modal elutriated fractions after induction of FV-P-infected JG6 cells with 210 mM DMSO in liquid culture. Only Trypan Blue viable cells were measured. By 48 hr. the modal shift has stabilized for the differentiated population.

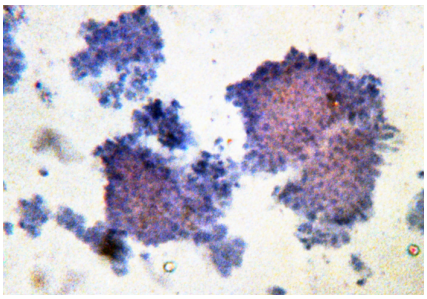

Fig. 11 (lower left) - Burst formation in a semi-defined serum-free medium by DMSO-induced Friend erythroleukemia JG6 cells grown in the absence of EPO in a 5% CO_2 atmosphere. Large, fully hemoglobinized bursts are apparent. Benzidine-Hematoxylin stain. Mag: ~25x.

sufficient, in the absence of DMSO, to induce them to extrude the nucleus (see **Fig. 12**) and undergo cell death in a manner similar to autoschizis, rather than the production of functional, hemoglobinized erythrocytes. Conversely, DMSO induction generated a substantial number of hemoglobinized CFU-E-like colonies and BFU-E-like bursts (**Fig. 11**).

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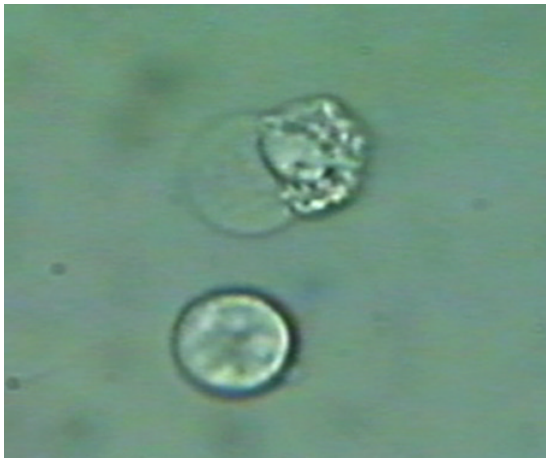

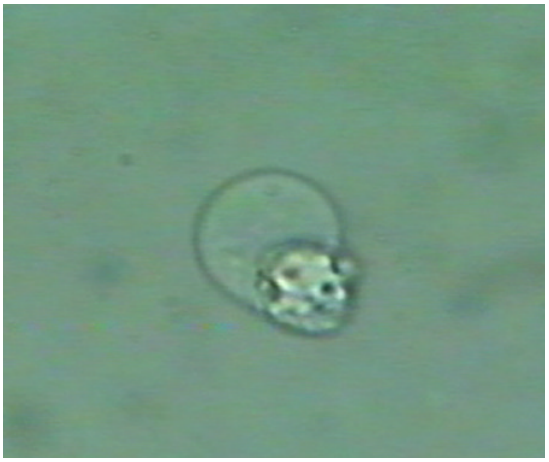

Fig. 12A (right) & 12B (left) - Friend erythroleukemia JG6 cells in liquid culture undergoing spontaneous nuclear extrusion during cell death induced by 7 hr. exposure to atmospheric oxygen, after previous growth in 5% CO_2. Note in **Fig. 12A**, a typical intact JG6 cell with a large nucleus. Up until the last 10 years, this process would have been considered as apoptotic, and seen as an extrusion of the nucleus in the process of its becoming pyknotic. But the work of Jacques Gilloteaux et al (Gilloteaux J et al (1998) *Scanning*, 20:564) suggests that the process is autoschizic if the cytoplasm excised into a bleb contains no organelles (thus it is the cytoplasm that is extruded in a bleb, a change of optics). However, one could observe intense motility of organelles left behind in the cytoplasm bleb, as characteristic of apoptosis. Yet, unlike apoptosis, the cytoplasmic bleb does not contain nuclear apoptotic bodies, nor is the cell reduced to small pieces. Rather, as in autoschizis, the nuclear envelope is intact, the nucleus undergoes progressive karyorrhexis and then karyolysis, and the cell proper is reduced to the perikaryon. Brightfield, 63x neofluar objective. Filmed with Hitachi VK-C2000 CMOS camera (270 lines). Mag.: ~1,800x.

Normal avian erythrocytes, like murine ones, retain their nucleus, and infection with AEV inhibits hemoglobin synthesis while promoting hyperplastic growth. AEV temperature-sensitive mutants could be shut down upon temperature elevation and the infected cells would resume hemoglobin production and the normal differentiation program with its limited proliferative potential [89]. The switch between transformation and differentiation found in Friend cells was, in avian erythroblastosis, revealed as a reversible process.

Singularly, the existence of a reversible switch between transformation and differentiation was raised in conjunction with the fact that these acute leukemia viruses often target (nucleated) erythroid progenitor cells. Yet, parallel transformation/differentiation switches could be observed in human cell lines not established by viral infection. The human erythroleukemia cell line K562 also demonstrates commitment to differentiate and, like FLC, differentiation in the absence of inducers [90]. As happens with late Friend-transformed cells or established Friend cell lines that are chemically induced to

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differentiate, one wonders whether the differentiation of the K562 cells is 'normal' or 'metaplastic'. Studies have shown that differentiated K562 cells have an heterogenous distribution of hemoglobin types, all embryonic and none adult [91]. Most remarkably in light of the next section, is that K562 cells seem to depend on IGF-I for their hyperplastic proliferation, and when induced to differentiate by hemin down express the number of IGF-I receptors [92], mimicking fetal erythropoietic cells and suggesting that their differentiation is likely metaplastic and not normal. Similar doubts arise regarding chemical induction of human HL-60 leukemic cells, which results in granulocytic differentiation [93]. In hemin-induced K-562 cells, RNA interference-mediated knockdown of the α-hemoglobin stabilizing protein (AHSP) decreases the production of fetal hemoglobin and increases apoptosis [94]. Given that the differentiation is abortive and ultimately kills the cell, K-562 cells have in recent years been used as a privileged testing ground for clinically-intended drugs that may induce cell death of leukemia cells. It is doubtful whether much can be learned about the transformation/differentiation switch from so many of these studies, since the forward switch mostly leads to apoptosis in response to induction by a great many agents. That the transformed phenotype is sustained in K562 cells by the oncogene fusion protein Bcr/Abl was confirmed once more when expression of the catalytically-active protein-tyrosine phosphatase receptor-type O (PTPROt, which is normally expressed in hematopoietic cells) inactivated Bcr/Abl proteins and reduced the phosphorylation of downstream targets [95]. This was followed by decreased proliferation, loss of anchorage-independent growth and inhibition of tumorigenicity, raising the possibility that suppression of PTPROt may be involved in K562 transformation. Altered nuclear response to retinoids may also be involved in maintaining the transformed phenotype in both K562 and HL-60 cells. The retinoid all-*trans*-retinoic acid (ATRA) has been a potent therapeutic agent in the treatment of acute promyelocytic leukemia (APL or AML-M3, see below) [96-97], and shown to be a co-inducer of erythroid and myeloid differentiation in serum-free studies of human BFU-E from normal peripheral blood [98]. Altered nuclear receptors for ATRA seem to play a key role in sustaining the transformed phenotypes of K562 and HL-60 cell lines since, through different nuclear pathways, an engineered retinoid (CD437) inhibits the proliferation of ATRA-resistant cells of both lines, and induces their apoptosis [99].

6. Oncogenes, viral transformation and the emergence of the molecular biology of growth factors

The failure to isolate mature viruses from most human tumors, together with the discovery that replication-defective retroviruses integrated into host chromosomes and could be silently transmitted across generations as a part of the host genome, led molecular biologists to initially assume that most, if not all, human cancers, whether they appeared to be familial or nonfamilial, were in fact

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genetic and inheritable. This model originated the notion that there was an inheritable predisposition to cancer caused by the infection of germ cells with replication-defective retroviruses, their vertical transmission establishing them as endogenous viruses. Irrespective of the existence of a genetic predisposition caused by heritable viral integrates, the absence of such silent integrates in most human cancers subsequently suggested that others forms of genetic and nongenetic predisposition must exist. Various genetic predispositions have long been studied in inbred mice as genetic susceptibilities to infection and transformation. Genetic control of susceptibility to infection with retroviruses was first studied with the spleen focus assay method for the FV-P in mice [100]. But nongenetic susceptibilities - such as caused by the dynamic physiological effect of tissue hypoxia, or hypoxia-like effects, etc - have been poorly studied.

Some susceptibilities might at first appear to be nongenetic, but subsequently reveal that they are the result of the absence of genetically encoded negative regulators that confer resistance to infection or transformation. Eventual identification of genetic FV-P resistance in B6 mice bred for the purpose showed this resistance was mediated by expression of a negative regulatory protein [101-102]. Other forms of resistance could equally be at work *in vivo*, in particular those involving the immune response and the adaptive genetics of its humoral component, or the cellular epigenetic control of DNA expression.

What was becoming apparent was that transforming viruses hit genes or gene complexes (loci) that were normally involved in the response to growth and differentiation signals (growth factors) that regulated, positively and negatively, cellular metabolism, proliferation and differentiation. It became evident that either altered regulation of the cellular proto-oncogene precursors of viral oncogenes, or the activation of genomically-inserted viral oncogenes from replication-defective retroviruses could be involved in causing malignant transformation of cells [103]. At the limit, however, no viral involvement was at all necessary, and this was in effect the situation with most 'wildtype' cancers. There was no universal viral etiology of cancer. Instead, a more profound link had begun to emerge between cancer and the biology of regulatory growth and differentiation factors.

The so-called cellular oncogenes were not cancer genes at all, but normal genes targeted by an oncogenic process that altered them. They were genes encoding not just growth and differentiation factors (gf's), but receptors for these gf's (the ligands), or for free soluble proteins that would bind these factors and modulate their concentration and delivery to receptors outside *and inside* the cell. As the biology of growth and differentiation factors became better understood, the class of oncogenes continued to expand to include a variety of signal transducers and DNA-binding proteins (for example the *myc* and *myb* group of genes) controlling gene expression. There were complex enzymic circuits of signal-transmission involved in normal cell physiology which are disturbed in oncogenesis,

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some working at the interface of a medium with the cell surface, others within the cell cytoplasm and on the nuclear membrane, and still others within the cell nucleus.

Initially, oncogenes comprised two classes - those encoding growth factor receptors and those encoding growth factors. Viral oncogenes frequently truncated the external domain of a surface receptor, on a region needed for binding a specific factor (*viz* the v-*erb-B* gene product of AEV and the epidermal growth factor receptor, EGF-R [104-105]), constitutively turning on the receptor and thereby rendering the cell independent of regulation by the physiological factor. Alternatively, in what is called "autocrine stimulation", viral oncogenes activated in the transformed cell generate a truncated ligand that stimulates a specific growth factor receptor expressed by cell itself (*viz* the v-*sis* gene vs the c-*sis* gene products and the receptor for platelet-derived growth factor, PDGF [106]). Since production of the substitute ligand is not physiologically regulated, but autocrine, the host cell is also rendered phenomenologically independent from the normal regulatory factor. Lines from CD19+ Pre-B cells of childhood ALL implemented both GM-CSF (Granulocyte-Monocyte Colony Stimulating Factor) autocrine and paracrine methods to achieve independence from growth factor control (the response of the G2 cell line is shown in **Fig. 13**) [107].

Blood is an organ (or subsystem) that is formed by the developmental separation (disjunction) of discrete cell lineages in a clone, with each clone being grown from a common blood-forming (hematopoietic) stem cell progenitor. Each blood cell lineage is developmentally and histologically regulated by a multiplicity of growth factors, growth factor receptors and signal-transducing networks [108]. Each factor or group of factors acts at a defined stage of development of a given hematopoietic lineage to promote progression through the cell cycle, mitogenesis and commitment to differentiate. One may therefore anticipate that, irrespective of any viral involvement, different neoplasia-promoting pressures would induce cellular independence from the factor or factors critical for the development and functioning of the blood cells most affected by those pressures. A case in point was the polycythemia variant of Friend virus (FV-P), which conferred infected cells hyperplastic growth and EPO-independent differentiation (EPO is the normal hormone responsible for the production of erythrocytes; it promotes commitment to terminal erythroid differentiation and sustains erythrocyte maturation). The virally encoded gp55 protein characteristic of the SFFV moiety of FV-P specifically targeted the EPO receptor [109], with which it binds intracellularly to make the cell independent from control by the physiological growth factor EPO. Effectively, gp55 mimicked the action of EPO on the EPO receptor, as if the receptor was constitutively turned on. Here was an envelope gene - *not technically an oncogene* - but which mimicked the hormonal ligand (part of its stereoscopic configuration) without emulating its gene (or gene sequence).

Still another example of how virally transduced oncogenes appear to selectively target growth

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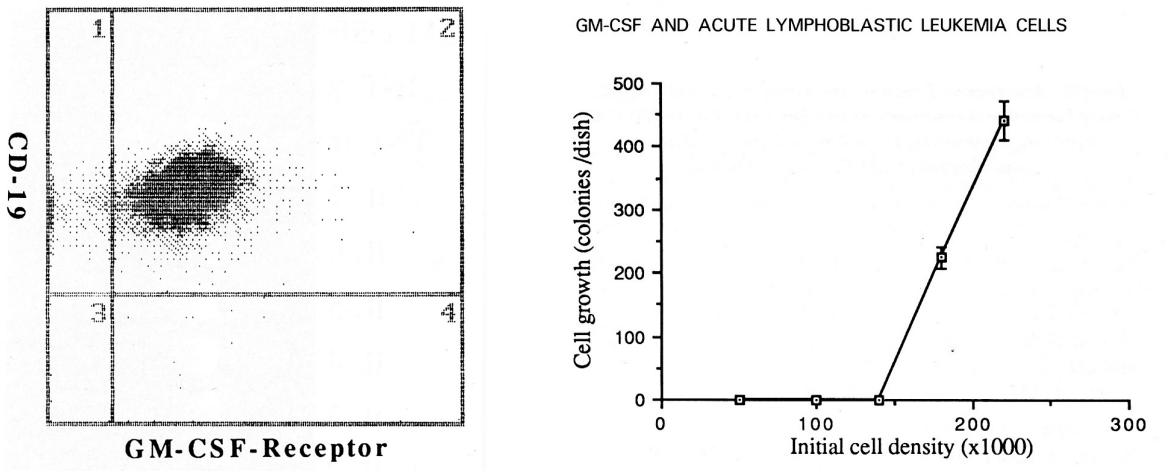

Fig. 13A (left) - Flow cytometric typing (cross detection) of GM-CSF receptors in CD19+ pre-B cells in ALL cell line driven by autocrine growth. 96% of the cells expressed GM-CSF receptors (after Freedman et al 1993 reference [107]). **Fig. 13B (right)** - GM-CSF autocrine growth of blast colonies from CD19+ G2 cells (pre-B cell ALL line) in serum-free culture (Correa & Axelrad 1991 reference [115]) and in the absence of any added growth factors, to observe the autocrine effect of cell density in blast colony formation (after Freedman et al 1993 reference [107]).

factor circuits with the selectable aim of making the transformed cell independent of hormonal control is the evidence for Abelson-MuLV abrogating the IL-3-dependence of mast cell proliferation by expression of the v-*abl*-specific transforming protein [110].

Whether oncogenesis had viral involvement or not, oncology came to discover that there was everywhere present a tendency on the part of the neoplastic process towards achieving cellular independence from normal tissue control and regulation by growth factors (the Leo Sachs hypothesis [111]). But as it slowly emerged after decades of investigation, full independence may be preceded by, or be the end-point of, varying degrees of hypersensitivity in response to growth factors, and independence from a given factor may also be a function of the hypersensitivity to another one. It is not a question of two steps - hypersensitivity and then independence, but of degrees of hypersensitivity to one or more growth factors that eventually culminate in independence from a normal regulatory control, whether this is achieved by autocrine mechanisms, the constitutive activation of cytoplasmic or nuclear receptors, or still by other means such as infection with a retrovirus, etc.

The biochemical nature of the specific growth factors and receptors affected in oncogenesis - together with the fact that expression of particular growth factor receptors is specific to different cell

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lineages or tissues and to their stages of development - largely determines the phenotype of the neoplastic or myeloproliferative disorder. In PV (Polycythemia *rubra vera*), the phenomenological independence from regulation by EPO [112-114] results from hypersensitivity to IGF-I (Insulin-Like Growth Factor I) which is characteristic of PV erythroid progenitor cells (see **Fig.s 14 and 15** comparing the differential sensitivities of normal and PV circulating erythroid progenitor cells to EPO and IGF-I) [114-115]. This could only be detected in a serum-free medium, since serum contains insulin and IGF-I, and serum-containing media mask the IGF-I hypersensitivity as EPO independence. Thus *Polycythemia vera* may be regarded as a hyperplastic disease (a true erythrocytosis) of erythroid

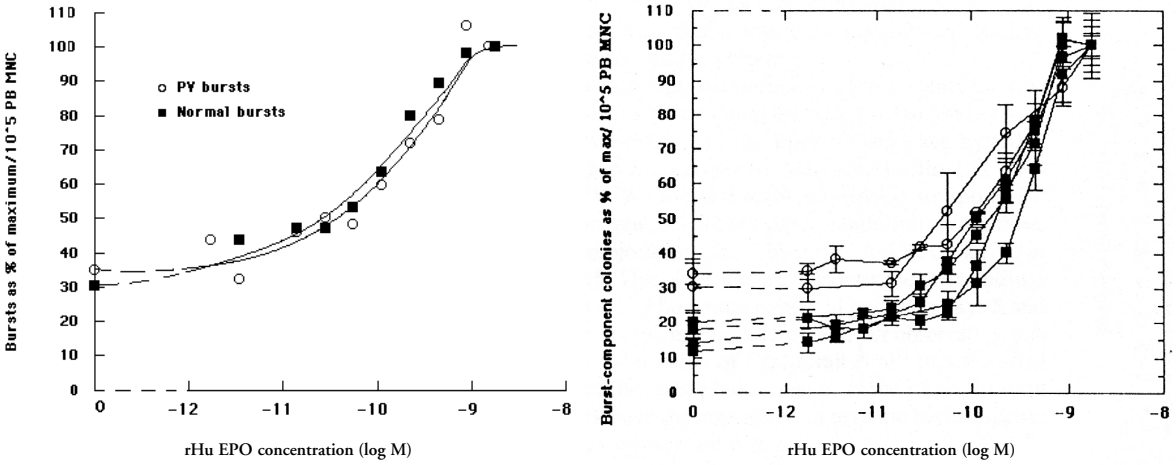

Fig. 14A - Normal vs PV erythroid burst formation by circulating erythroid progenitors in serum-free medium as a function of the rHu EPO concentration, in the absence of IGF-1 and expressed as a percentage of maximal EPO stimulation (after Correa 1991 reference [113]). All cultures contained plateau concentrations of rHu IL-3. **Fig. 14B** - Corresponding normal vs PV burst-component colony (BCC) formation for 2 PV donors and 4 normal ones, tested as described for Fig. 13A, and also expressed as a percentage of maximal EPO stimulation (after Correa & Axelrad, 1994 [114]).

precursor cells characterized by phenomenological independence from EPO and by hypersensitivity to IGF-I. In **Fig. 16**, we show how the IGF-I hypersensitivity is specific to PV and not to secondary erythrocytosis.

Growth factor hypersensitivity appears to play a dominant role in the chronic myeloproliferative disorders (CMPDs). In JCML (Juvenile Chronic Myelogenous Leukemia) and JMML (Juvenile Myelomonocytic Leukemia), the disorders are mediated by hypersensitivity to GM-CSF (the normal regulator of granulopoiesis) on the part of granulocyte and macrophage precursors [116-117]. In ET

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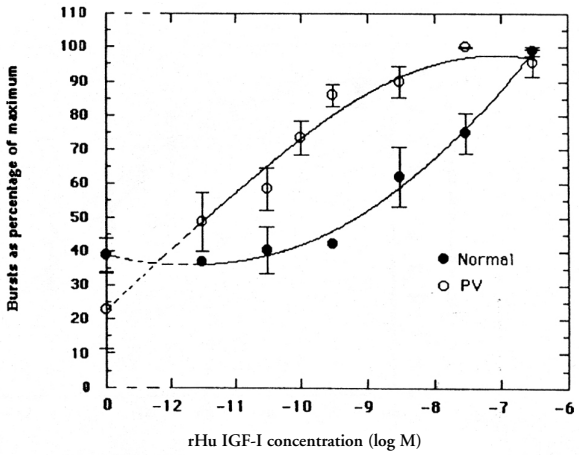

Fig. 15 - Comparison of erythroid burst formation by PV and normal circulating erythroid progenitors as a function of the concentration of rHu IGF-I in serum-free medium, in the absence of EPO, and expressed as percentage of maximal IGF-I stimulation. Data were pooled from 5 PV patients and 3 normal donors. All cultures contained plateau concentrations of rHu IL-3 (after Correa & Axelrad 1994 [114]). Contrast the IGF-I hypersensitivity of PV to its normal response to EPO shown in Fig.s 14A & B.

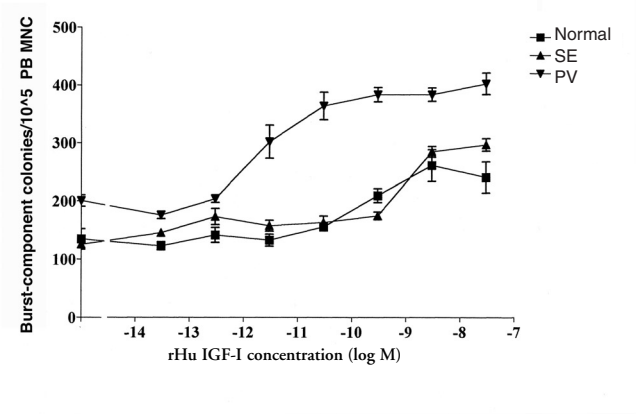

Fig. 16 - Comparison of IGF-I sensitivities of circulating erythroid progenitor cells in PV and secondary erythrocytosis, as a function of the concentration of rHU IGF-I in serum-free medium, in the absence of EPO. Closed squares: normal control; upward closed triangles: secondary erythrodytosis; downward closed triangles: PV.

(Essential Thrombocythemia), another CMPD, the hyperplasia is mediated by hypersensitivity to MGDF/TPO (thrombopoietin) on the part of megakaryocytic precursors [118-119]. And in IMF (Idiopathic Myelofibrosis), by SCF (Stem Cell Factor or KIT ligand) hypersensitivity on the part of the same cells [120]. These hypersensitivities are summarized in **Table 1**.

We suggest that these CMPD hypersensitivities that trigger and sustain the hyperplastic proliferation of diverse hemopoietic progenitor cells constitute an initial phase of the oncogenic process in the blood system. At initiation, the neoplastic disease vector responded to specific selection pressures, and did so without any viral involvement. In an early phase - a 'pre-neoplastic hyperplastic stage' - there was establishment of a myeloproliferative disorder mediated by tissue- and development-specific hypersensitivities. The early stages of the oncogenic vector still responded, but dysfunctionally, to organic and tissue constraints. Though engaged in oncogenesis and thus transformed to an extent, these cells were not yet fully independent of the hormonal circuits of the organism.

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Table 1
The cytokine hypersensitivity hypothesis

Myeloproliferative Disorder	Progenitor Cells	are specifically hypersensitive to
PV	erythroid (BFU-E)	IGF-1
JCML/JMML	granulocytic	GM-CSF
ET	megakaryocytic	MGDF/TPO
IMF	promyelocytic (CFU-GEmM)	SCF

Accordingly, at first, the oncogenic vector targeted recursive and hypersensitivity hormone mechanisms capable of sustaining a proliferative disorder. This may well be a universal strategy of the oncogenic process, inasmuch as it propagates the initial mutation and thereby increases the number of cells that can be targeted for further transforming mutations (curiously enough, this is an argument that does not apply to PV, since its further evolution into leukemia apparently does not emerge from the PV clone proper).

In this sense, it is furthermore tempting to regard PV and its IGF-I hypersensitivity as an anaplastic regression to an embryonic state when erythroid proliferation and differentiation was driven by IGF-I, the fetal erythropoietic hormone [113]. Just as it is tempting to see IGF-I independence as the next step in neoplastic development *with respect* to the IGF-I circuit that controls both proliferation and differentiation. Indeed, cultured cells - presumably sarcomagenic - derived from human cells established by transformation with transfected c-*src* mutants constitutively activated (tyrosine-phosphorylated) their IGF-I receptors, and no longer required addition of IGF-I for proliferation [121]. A variety of truncations in the extracellular sequence of the IGF-I receptor gene transduced by an ASV vector, correlate with the degree of tumorigenicity and the extent of tyrosine phosphorylation of an array of cellular proteins [122]. Down regulation of a docking protein that mediates the IGF-I interaction with its receptor in v-*src* transformed cells reverses their transformed phenotype, including IGF-I-independent growth in serum-free medium [123].

In the context of a potential pivotal role for the IGF-I axis in the unfolding of the oncogenic vector, it is also noteworthy that a variety of transformed cell lines are autocrine for IGF-I, which can

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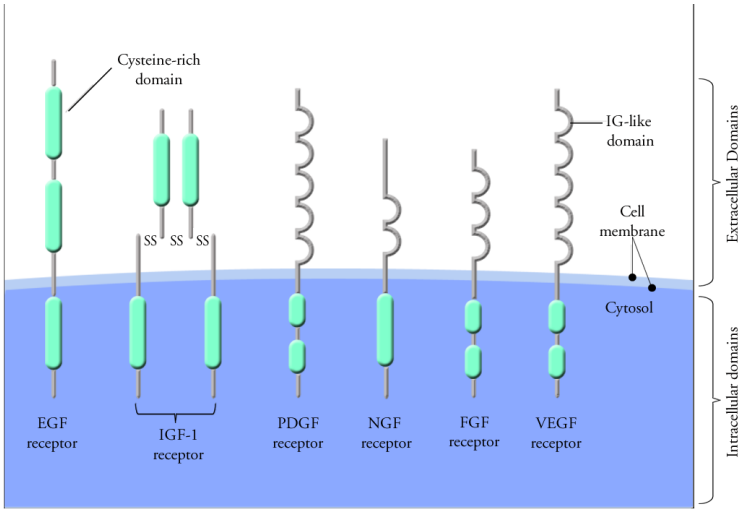

Fig. 17 - Growth factor receptor tyrosine kinases with cytoplasmic domains. EGF, Epidermal growth factor; IGF-I, insulin-like growth factor I; PDGF, platelet-derived growth factor; NGF, nerve growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor. SS denotes disulfide bonds.

be seen as an alternative, recursive path towards independence from hormonal regulation. Epidemiological data suggests that chronic hyperinsulinaemia and low IGF-I plasma levels are causatively linked to cancers of the colon, pancreas and endometrium, and possibly of the breast, whereas high levels of IGF-I (as net concentration or relative to IGF-I binding protein 3, IGFBP3) seem to correlate with increased risk of prostate cancer, rectal cancer and breast cancer in young women [124]. Thus aside from IGF-I hypersensitivity in PV, it seems that the insulin and IGF-I circuits are implicated in a variety of other oncogenic and tumorigenic processes [124-125], because of their role in sustaining increased proliferation by promoting catabolic reactions and controlling energy metabolism, including the expression of hypoxia-inducible genes. The IGF-I receptor (IGF1R) involved in PV hypersensitivity [113-114, 126] is crucial for tumorigenicity and the proliferation of tumor cells, and its signaling interferes with, and overrides, other growth factor/receptor circuits - not just those controlled by EPO in erythroid progenitors, but also those mediated by vascular endothelial growth factors (VEGF) and the EGF receptor (EGFR). VEGF, which controls normal angiogenesis, is the factor employed by tumor cells to vascularize the tumor.

It is most remarkable that activation of the tyrosine kinase receptors (see **Fig. 17**) for EGF, IGF-I, PDGF, FGF (fibroblast growth factor), VEGF and NGF (nerve growth factor) is found to be involved in a variety of human cancers [127-129]. Deletion-induced constitutive activation of the EGFR and activating mutations in other members of the EGFR family (including KIT, the receptor for SCF) have been shown to occur in lung and breast cancer, as well as in gastrointestinal stromal tumors carrying a KIT mutation [130-131].

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The study of the viral induction of cancer had led to the discovery of cellular proto-oncogenes, and this, in turn, to the discovery of the molecular biology of growth factors and the signal effector circuits encoded by these cellular oncogenes - ie to the discovery of the precise molecular domain of the biological regulation of the whole in each of the parts. Cancer now began to appear as a disease of the deregulation of normal regulatory factors, a dysfunction of growth factor biology. All the growth factors were mitogens, but these mitogen signals controlled and limited proliferation often as a function of a commitment to differentiate and terminally proliferate. Independence from the regulatory mitogens concomitant with constant activation of the signaling circuits operating downstream from the mitogen receptors then resulted in the maintenance of an indefinite state of rapid proliferation.

Families of growth factors and their receptors were involved in a variety of organs, in their development and physiological functions, and each specific receptor intersected a multiplicity of intracellular signaling pathways, not just 'its own' [132]. Epidermal growth factor receptors were critical mediators of intracellular communication and phosphorylation of intracellular signal-effectors in kidney, liver, skin and other organs, and invariably their action was modulated downstream by converging pathways, both synergistic and antagonistic [133]. Moreover, evidence began surfacing of crosstalk between different receptor families and circuits - as between receptor tyrosine kinase and receptor serine/threonine kinase pathways - and of receptor turn-off controls exerted by cytosolic protein tyrosine phosphatases. Individual signaling events were intersections of multiple pathways in a cellular network. Cellular control was the result of a multiplicity of modulations. No cellular functions were linearly determined, even in "proportional responses".

The picture of the cancer cell that then began emerging and slowly coming into focus was that of a kind of 'histological anarchist', a cell engaged in what we shall call an "amoeboid-becoming" and enjoying a molecular freedom from the organism: cancer was a dissolution of the histological and cellular order of tissues and an alteration of cellular metabolism and physiology. It was a pathophysiological process operating within the cell, by transformation, and outside the affected cell, by progression of the transformation vector to tissue invasion and tumor formation. The cancer cell liberated itself from the histological and physiological constraints of the organism - the very constraints that were molecularly embodied by the action of regulatory growth factors. The oncogenic process targeted precisely the regulatory circuits controlled by these factors. Utilization of viral vectors was relevant for transformation only so long as the vectors in question were able to target the same regulatory circuits that were under pressure. Cells could just as well engage an oncogenic process without mediation by viral vectors. What was new and important was the realization that the growth of a neoplastic cell was no longer modulated by normal hormones or growth factors, no longer part of a

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collective web of tissue self-regulation. The replication and proliferation of the cancer cell was no longer controlled as part of a differentiation program and subject to feedback control and contact inhibition. It had abandoned the normal differentiation program, and reversed this to a degree (anaplasia). When induced, its 'differentiation' was metaplastic, often mimicking embryonic or fetal differentiation. It had abnormally increased its rate of glycolysis, and did so anaerobically, at first facultatively, then obligately. It eventually acquired motility and was able to migrate and invade tissue. By a variety of genetic processes, the neoplastic cell had acquired mutations in complex arrays of oncogenes and other types of genes that permitted it to shirk off critical controls of metabolism, proliferation, differentiation and contact inhibition.

7. The demise of viral oncogenesis as universal etiology of cancer and leukemia

The demonstration that both DNA and RNA viruses could induce neoplasia in animals and malignant transformation of cells (primary or immortalized) in tissue culture did not so much unify the medical and scientific understanding of the etiology of cancer, as it left a whole new set of unanswered questions. In an unpublished paper dating from the early 1960's, Axelrad enunciated all the pertinent questions that the discovery of viral oncogenesis had raised in its early days:

"The questions we must have answers to are: Do viruses play a role, say as agents in the genesis of all malignant tumors along the final common pathway to malignancy into which funnel all other means of tumor induction? Are viruses merely another kind of agent that causes mutation leading to cancer? Do they simply go along for the ride in the cell once they have done their genetic damage? Or do they continue to play an active role in the malignant cell and all its progeny; would the latter still be a malignant cell if it were freed from it? (Are some cells freed from it by asymmetric division?). Do they continually stimulate proliferation when present in the cell? Do they continually interfere with the normal flow of information to and from the genetic apparatus, thus perpetuating an inability of the cell to respond to cues from other cells that tell the cell when to divide and when not to? Could the action of the virus conceivably increase the probability of errors occurring in the genetic material or its means of accurate replication or distribution to daughter cells?" [134].

In articulating a tentative response to these questions, Axelrad made a profound suggestion relating oncogenic induction and the type of tumor produced to the adaptive pressure that selects for a particular transformed genotype:

"Continued presence of the virus could account not only for the unresponsiveness of malignant cells to growth-controlling influences, but also to loss of their inherited differentiated character, loss of specific

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antigens, perpetuation of the tendency to mitotic irregularities and thus for the possibility of the continued production of cells with new genotype from which natural selection could then determine the character of the tumor." [134]

Almost five decades later, most of Axelrad's questions received an answer.

The genesis of most tumors is not viral, nor virally induced. Viruses are - like mutagens and other micro-organisms, namely mycoplasma [135] - merely promoters or mediators of co-related switches in metabolism, growth and differentiation that engage an oncogenic process (they merely play a role along the same pathway that funnels the action of all the inducers of malignancy). In oncogenesis, there is always a multiplicity of molecular factors at work - even if they focus at first on a given regulatory factor circuit. But here the changes, too, are diverse and involve a multiplicity of other regulatory circuits, as the oncogenic process alters the overall cellular network composed by all separate but interlinked regulatory circuits. Likewise, we encounter a multiplicity of factors, biological and molecular, at work in the induction of cancer - not just viruses. There are various DNA viruses, retroviruses, mycoplasma, and even Gram-negative bacteria that get in on the action, nearly always surprisingly. For example, the spleen focus-forming efficiency of SFFV in mice can be substantially enhanced by prior exposure of the target cells to lipopolysaccharides excreted by Gram-negative bacteria [136]. It is always a multiplicity of cells *and* viruses *and* biologically active molecules at work, an heterogenous multiplicity or a multiplicity of elements from different classes, species or types of 'biological objects'. In fact, it was long ago observed that cells could be double transformed by superinfecting them with two viruses, one a DNA virus, such as SV40, and the other a retrovirus, such as a murine sarcoma virus [137] or the Rauscher leukemia virus [138]. Instead of competition, there are degrees of transformation corresponding to the synergisms of various acting multiplicities or biological assemblages - at once at the level of cells, micro-organisms and viruses, *and* at the molecular level, where the multiplicity is effectuated by a variety of regulatory and signal-transducing enzymes.

Moreover, aside from the fact that most often there are no distinct agents, but "acting biological multiplicities" that are tiered and coupled together in networks, one should keep in mind that expression of endogenous viruses and susceptibility to infection upon exposure to viruses is now known to depend on both genetic and dynamic, nongenetic factors. Foremost among the latter is hypoxia (to which we will return in detail in a follow-up communication [139]), which enhances infection with leukemia viruses (eg FV-P, FV-A, AEV) that infect erythroid progenitor cells. Thus one must keep in mind that susceptibility to viral infection is not simply determined as a genetic susceptibility. Other factors - dynamic ones that relate to the composition of biological multiplicities - enter in the determination of cellular susceptibility to viruses.

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Viruses are not causative agents of cancer. A virus only functions as any other mediator does in the oncogenic transformation of a cell - as an element that affords the cell the possibility of amoeboid-like adaptive changes. But a virus functions as such an element because indeed it presents the transformed cell with an adaptive advantage - the possibility of a "continued production of cells with an altered genotype from which natural selection can then determine the character of the tumor", to paraphrase Axelrad above. Yet, this does not preclude cells from undergoing comparable adaptive changes without viral mediation. These changes target genes involved in the regulation of all the fundamental activities of a cell. And cells engaged in an oncogenic process seem to alter these genes by very different pathways.

The viral oncogene theory of cancer is the apogee of all XXth century viral theories of cancer. But it failed to account for the majority of known human cancers. Central to the change in perspective about the etiology of cancer not being necessarily viral, were findings that indicated that transformation caused by altered oncogene expression could indeed be brought about by a variety of genetic changes. Some *were viral* mechanisms - such as *transduction of viral oncogenes* that belong to an inserted retroviral genome (eg v-*src*); *insertional activation of cellular oncogenes* by integration of viral sequences carrying a strong promoter or enhancer adjacently to the cellular oncogene sequences that they activate; or *insertional mutagenesis* that alters normal expression of cellular oncogenes. But other genetic changes *were nonviral*, and either simulated viral transduction in the absence of any viral involvement (such as gene transfer of oncogenes from tumor cells to normal cells) or involved chromosomal abnormalities (see **Table 2**) - such as: chromosomal translocations at breakage points that are veritable hotspots, large deletions, or inversions that result in oncogene activation; or supernumerary chromosomes (often called "double minutes" when they lack centromeres) that result in gene amplification. Rather than cancer having a universal viral causation, the autonomous genetic changes of transformed cells indicated that the oncogenic vector constituted a potentiality inherent to every cell that had not terminally differentiated.

Aside from the reservations now put to studies of transformation conducted with established cell lines - and not to mention the fact that most such investigations still continue to employ chemically undefined media that are exposed to serum contaminants - it is apparent *in vivo* that certain viruses may promote specific malignancies, but viral promotion is not sufficient on its own and requires association with either chemical and physical mutagens, or with other micro-organisms, as well as specific genetic and dynamic susceptibilities to viral infection. Thus, in general, viruses appear to be *neither necessary nor sufficient* for oncogenesis. Whereas in the 1980's one often heard teaching oncologists and virologists complaining that medical doctors were reluctant to accept the viral basis of all cancers, and that, in all cases, there was an hereditary predisposition to it, today no self-respect-

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ing oncologist would be caught dead making such pronouncements, especially when cancer encompasses more than 100 different types, each with many variations, and no single parameter or principle can function as the rational explanation of a unity behind their diversity. To many, this has suggested that cancer is not a single disease, thereby precluding the notion of a single cure [140].

The final nail on the coffin of the viral oncogene theory of cancer - what dashed its hopes that all manifestations of cancer would prove to have a viral causation - was the inability to isolate viruses or identify active viruses integrated in the host genome in the great majority of human cancers and tumors. Only two human malignancies appear to have 'a viral etiology', or a definitive viral involvement in their etiology: Burkitt B-cell lymphoma induced by EBV, characterized by three translocations, one of which places the IgH enhancer near the cellular oncogene c-*myc* in transformed lymphocytes [141-144] (see **Table 2**); and adult T-cell lymphomatic leukemia (ATLL, or ATL as it is called nowadays), which is endemic in southern Japan, the Caribbean basin and Central Africa, and associated with HTLV-I infection and expression [145]. Breast-feeding is the presumed vehicle for the vertical transmission of HTLV-I. Perhaps there is a third human malignancy, carcinomatous in nature, that also has viral involvement - since some strains of human papilloma virus (HPV) may be required in the induction of cervical and other epithelial carcinomas, as was already discussed above.

We should note in passing that, leaving aside viral induction in Burkitt's lymphoma and ATL, there are to this day only four unambiguous known causes of leukemia: ionizing radiation, benzene exposure, chemotherapy (at last an admission on the part of medical researchers!) and rare inheritable syndromes [146]. Yet most 'wildtype' cancers are not familial, nor induced by viruses or specific mutagens.

The school of the viral etiology of cancer succeeded in experimentally demonstrating that viruses can succeed in inducing sarcomas and leukemias. But as the investigation progressed and became ever more sophisticated, it became clear that the smashing majority of cancers did not have a viral etiology, but rather entailed a multiplicity of variable genetic changes that altered cellular metabolism, growth control and differentiation, and which were autonomously developed as newly acquired lamarckian characteristics. We are here reminded of the caution once uttered by Lwoff in his address delivered on the occasion of Otto Mühlbock's 65th birthday: he reminded one that "there is, in short, an important distinction to be drawn between cancer as the response to a contagious, infective agent of the conventional type, and cancer as a process of aggressive cellular growth due to the inheritance [transmission] of new characteristics that follows modification by mutation of elements that may indifferently be small and filterable or large and nonfilterable" [our translation], ie be these new genetic traits conveyed by viruses or instead by gross chromosomal alterations or mutations with no viral involvement. As the understanding of viruses progressed during the last 5 decades in both

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virology and oncology, it became evident that infection - with viruses or other agents - played only a very minor role in the etiology of 'wildtype' cancers. As this realization dawned, 'wildtype' cancers became understood as the result of diverse processes of directed neo-lamarckian experimentation in the acquisition of new inheritable characteristics (regarding hormone response pathways, metabolic switches, growth and differentiation) that respond to systemic neoplasia-promoting pressures [41]. The cancer cell was now seen as a laboratory of molecular experimentation with new characteristics selected from the adaptive modification of genetic elements [147-148].

Accordingly, cancer became conceptualized as an adaptive disorder of growth factor biology [128] and energy metabolism (for a review see [139]). And thus the concept of the oncogene had to shift once more - as it once had shifted from a viral to a cellular gene with the proto-oncogene theory; or later had gained definition when it became the concept of a gene encoding regulatory factors and their receptors controlling cell metabolism, proliferation and differentiation. Now, it had to expand still further to include membrane-associated and cytoplasmic signal transducers, DNA transcription factors, apoptosis regulators (eg *bcl-2* gene) and, more recently still, 'chromatin remodellers' and microRNA genes (more on this below).

8. The oncogenic vector and the differentiation/transformation switch revisited

One of the critical outcomes of the oncogene theory of cancer was the realization that the phenotypic markers of malignancy relate to the failure of normal cells to respond to the so-called 'Go' and 'Stop' signals. The switch occurs typically along a primitive differentiation pathway, during the proliferative phase, and is often marked by hypersensitivity to a given growth factor (which may be masked by independence from another factor), or by independence from it, or still by independence from a different growth factor having a regulatory action upon the pathway of the first one. The first steps towards malignant transformation most often arise in cells that are engaged in a normal proliferative state. This tallies with the older and more general notion that in leukemia there is progression between earlier chronic forms that target more mature hematopoietic progenitors and originate 'cytoses' (eg the erythrocytosis of PV, or the granulocytosis of CML, etc), and later acute forms that target more primitive progenitors and give rise to 'blastoses' (eg the evolution of both PV or CML into acute leukemias).

As already discussed, neoplastic transformation - or the oncogenic vector - has been seen as a layering process involving initial and progression events. Fully developed cancer cells often present gross genetic changes, not just discrete genetic mutations. Gross chromosomal abnormalities have been detected in practically all cells from tumors and many leukemias (see **Table 2**): entire chromosomes are lost, or duplicated a number of times; chromosomal translocations are frequent, and so are

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deletions of entire chromosomal regions; pieces of chromosomes are fused together to form abnormal chromosomes; genomic sequences are scrambled, swapped, extended or inverted. Gene mutation and gene fusion - caused by translocation, inversion or deletion, as well as by juxtaposition of enhancer elements - have been historically seen as events that can initiate malignant transformation of target cells and also that may sustain further progression [149-152], whereas DNA amplification is seen as an event characteristic solely of progression (for a review see [129]).

How are these cancer initiation and progression events related to the potential for differentiation on the part of malignantly transformed cells? Leo Sachs' demonstration that addition of the normal physiological growth factor elicited granulocytic differentiation and loss of the properties of transformed cells in myeloid leukemia [111, 153] suggested that a full circle had been reached: transformation arose by a switch in the differentiation pathway that ultimately led to independence from a growth factor, and transformation could be reversed in some cells by induction of differentiation with physiological growth factors.

But was this the complete story? Were these revertants normal cells? Can induced differentiation of transformed cells actually be conceived as a reversion to the normal program? What to say of induced differentiations that are unrelated to the differentiated state of the tissue which became transformed? How do HeLa cells from a human cervical carcinoma yield erythroid bursts in clot culture stimulated with serum and EPO (see **Fig. 18)?** Could a sufficiently high gradient of a normal growth factor induce normal differentiation of all transformed cells sharing the same phenotype? And do so for every type of tumor cell? As mentioned already, the evidence with Friend cells and human myeloid leukemia lines that were induced to differentiate with a variety of molecules, including normal growth factors, indicates that the differentiation is not normal. Further, that, as Friend disease progresses and tumorigenic cells emerge, the inducibility of differentiation decreases and disappears. It would be only the hyperplastic stage of the oncogenic vector that presented inducibility of differentiation. Generally, the induced differentiation partook of embryonic characteristics - in line with the observed activation of fetal genes in established erythroleukemia cell lines, and the observation that a portion of the RBCs in PV carry fetal hemoglobin.

These facts suggest that the preneoplastic hyperplastic phase of the oncogenic vector corresponds to the immortalization of cells that permits the establishment of lines in tissue culture - during which phase the cells can still be induced to differentiate with varying degrees of approximation to physiological normalcy. The cells are transformed already, but not neoplastically. They may be tumorigenic (like papillomas or embryonal carcinomas are), but are not able to metastacize or invade tissue (to behave like amoebae and break through basement membranes), and thus their tumors are benign.

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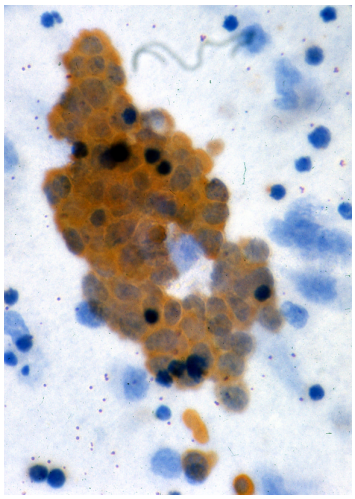

Fig. 18 - Human cervical carcinoma HeLa cell-derived erythroid d10 burst in plasma-clot culture induced with EPO. Mag,: ~225x.

Is this hyperplastic phase always benign? Seemingly it is both benign and pre-neoplastic, and thus already in some way part of a transformation that is oncogenic or leads to malignancy. Or, to put it differently, at some point or under certain conditions it ceases being benign to become malignant, but reversibly so. In the instance of teratocarcinomas, specifically with most of their subpopulation of embryonal carcinoma (EC) cells, the tumors seeded by these stem cells - after forming a number of *malignancies by transplantation from host to host* - suddenly stop growing, stop being transplantable, and instead form benign tumors known as teratomas, where they undergo multiple differentiations that are in all respects normal save for the disordered state in which they arise (no systemic or 'organismic' body plan). It is evident that, in cases like this, the differentiation program has been genetically and phenotypically - though not histologically - recovered. So were the EC cells malignantly transformed or not? How do they seed transplantable malignant tumors and then begin seeding benign ones instead? If transplanted, they may kill the host like a malignancy does; yet it seems that EC cells are not neoplastic, but simply under epigenetic control because they retained the embryonic totipotent state characteristic of early cleavage stages, including a proliferative ability comparable to that of neoplastic cells. The fact that EC cells have a normal complement of chromosomes and are genomically stable (for a review see [154]) should, in fact, preclude their being considered the equivalent of neoplastic cells.

The most challenging of recent findings regarding the relationship between malignant transformation and differentiation is acute promyelocytic leukemia, APL (AML-M3), which is caused by translocations that result in enduring repression of a nuclear transcription factor, and thus block all-*trans*-retinoic acid (ATRA) induced differentiation. Besides being a vitamin A derivative, ATRA is a growth factor required for the differentiation of monocytic, granulocytic and erythroid lineages [155-156]. Whereas it is a permissive factor in the nonerythroid myelopoiesis, it is only a potentiator of EPO or IGF-I in erythroid differentiation [156]. The most prevalent of the translocations - t(15;17)(q22;q21) - that fuses the *PML* (promyelocytic growth suppressor gene [157]) gene with the

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gene for the ATRA receptor RARa in chromosome 17 - presents a *nearly-independent hypo*sensitivity of RARa to the ligand ATRA, sufficient to keep bone marrow myeloblasts in a state of indefinite proliferation or hyperplasia. But treatment of most APL patients with supra-physiological doses of ATRA is able to achieve remission and normal ATRA response, and is successful when it also eliminates the abnormal karyotype by inducing differentiation of all the cells of the malignant clone (for which purpose, ATRA by itself may not suffice [158]). Once the block to differentiate is lifted and the abnormal clone eliminated, normal granulocytic and monocytic cells are again produced by marrow.

Here we have a leukemia that presents all the critical telltale signs of malignant transformation - independence from a regulatory growth factor that results in hyperplasia and failure to differentiate, altered transcription of DNA by a gross karyotypic change (a translocation), even neoplastic tumorigenicity (shown with APL-derived cell lines in nude mice) and mutagenic suppression of a suppressor gene, *PML* [157] - all this with no viral involvement, and yet the same leukemia can, in most instances, be forced to recede and yield normally differentiated cells by exposure to massive doses of the normal regulatory growth factor. Some variants of the *RARa* gene fusion in APL yield instead differentiation-refractory clones that do not respond to growth factor therapy with ATRA - but in some cases respond to other retinoids, even engineered ones [99].

These findings suggest that the relationship between transformation and differentiation is a graded switch, and that even acute leukemias can be reversed by proper clinical use of the switch. One may wonder what is the wider significance of the blastosis in APL, and whether this does not contradict the notion that acute leukemias are late stage malignancies (according to the vector model: cytosis->blastosis). Perhaps a better notion is that the oncogenic vector progresses from (1) transformed states that are susceptible of being switched back to normal differentiation and are rarely tumorigenic or, if tumorigenic, are benign, to (2) transformed states that are capable of yielding quasi-embryonic and metaplastic differentiations and are typically tumorigenic and neoplastic, and lastly to (3) transformed states that are refractory to differentiation, highly tumorigenic, invasive and capable of metastacizing. At some advanced point in the oncogenic vector the differentiation switch is turned off entirely in transformed cells. The most tumorigenic of transformed cells then have in common their complete independence from physiological regulation of tissue metabolism, growth and differentiation. They and their progeny have become a totally foreign tissue, genetically and phenotypically.

The progression of the oncogenic vector thus appears to be related to how refractory a transformed cell is to differentiate. It seems that supernumerary chromosomes and, in general, DNA amplification are involved in this progression, and thus in barring differentiation altogether. Genes from four different oncogene families (c-*myc*, c-*ras*, cyclin D1 or *CCD1*, and *EGFR*) have been found

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to be amplified in carcinomas of the lung (small cell), breast, esophagus, ovaries, cervix and head and neck [159-161]. But other processes are equally at work in the progression of cancer, such as chromatin remodelling. In acute lymphocytic and acute myelocytic leukemias (ALL and AML), chromatin remodeling (via acetylation, deacetylation and methylation of nucleosomes and free histones) appears to play a major role in the generation of the chimeric ALL1(MLL) fusion proteins that deregulate homeobox genes (which encode transcription factors) and microRNA genes [162]. Translocations also plays a role in progression, as an additional t(9;22) translocation converts chronic myelogenous leukemia, CML, into an acute leukemia [128].

9. From oncogenes to the adaptive genetics of cellular auto-oncogenesis

By the mid-1990's, the revised oncogene/tumor-suppressor gene hypothesis had also failed. It had been unable to identify a particular pattern of genetic mutations in all the variants of any of the most frequent or the most 'virulent' types of human cancer - let alone any universal or quasi-universal process unifying our understanding of all types of cancer, and limited to the involvement of just oncogenes and tumor suppressor genes. This forced open the door to consideration of other models for the etiology of cancer.

Historically, one needs to understand that theories of viral causation of cancer have coexisted with other models, both in official science and in established medicine. The oldest theory suggested that cancer resulted from increasing gross genetic instability. In the 1970's a different model emerged which proposed that inhibition of tumor-suppressor genes caused initiation. This coexisted with a distinct model of the negative physiological regulation of proliferation - in which it was suppression of negative regulators that resulted in hyperplastic states. Dysfunction of the DNA repair genes was also suggested to play a causative role in the initiation of cancer. Fast accumulation of random mutations could eventually lead to activation of oncogenes, and the UV-induction of skin melanoma was invoked as a model. More recently still, the hypothesis of master genes was used to suggest that their inhibition causes cancer, or even more simply that cancer is the result of aneuploidy.

Gross chromosomal abnormalities have long been detected in cancer cells. They were, in fact, one of the first indications that genes reside in chromosomes. The presence of many mitotic figures in tumors alerted researchers early on to the likelihood that the fast rates of division of tumor cells meant they had a greater chance of accumulating chromosomal abnormalities (see **Fig. 19**). In 1914, one year after Rous' discovery of the involvement of RSV in avian sarcomas, Theodor Boveri suggested that either inherited or spontaneous chromosomal malformations could be the cause of cancer. To this day, we still do not know exactly what causes these aberrations. Their absence from germline cells suggested to oncology researchers and geneticists that cancer was the result of "somat-

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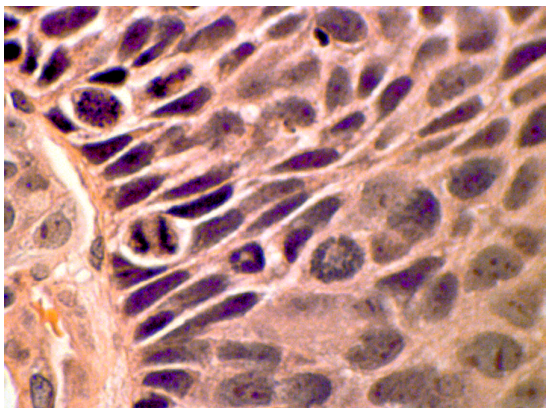

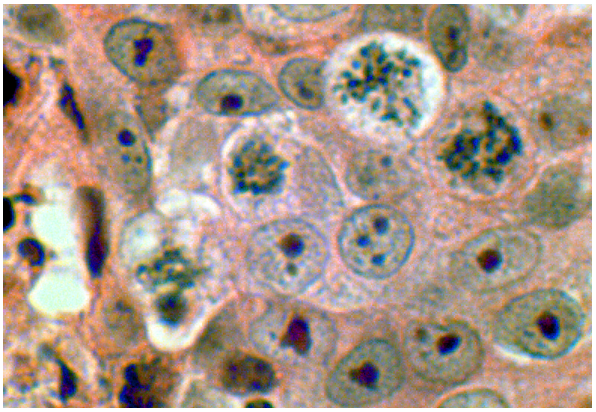

Fig. 19A - Atypia, spindle cells and mitotic figures (metaphase plates and anaphase) in a slightly invasive carcinoma (Luminera Infinity-1 camera, 450x mag.). **Fig. 19B** - Anomalous mitotic figures, including supernumerary chromosomes, in a carcinoma (Luminera Infinity-1 camera, 950x mag.).

ic mutation" [163]. A few persistent chromosomal breakage syndromes - eg Fanconi anemia, ataxia telegiectasia, Bloom syndrome, Rothmund-Thomson syndrome - are associated with an increased risk of neoplasia, and leukemia in particular, and they are either congenital (present in germline cells) or the result of an hereditary propensity for chromosome breakage. But most cancers and leukemias show heteroclitous amounts and variations of chromosomal aberrations that were not transmitted by germ cells, and without any apparent unity to them. The role these aberrations play in the genesis of cancer is still unclear. They may well be unnecessary for cancer initiation and not even the result of a selection operating on primary adaptive changes. More likely, they are the result of secondary selections involved in cancer progression, but not required for progression as such. The absence of a significant viral etiology to human cancers and leukemias, together with the presence of a myriad of chromosomal aberrations that hit recurrent gene loci (and thus increasingly appear not to be randomistic despite their diversity) led more recent alternative models of neoplasia to focus on studying these abnormalities, rather than on gene mutations *per se*.

The only human leukemias that present recurrent gross chromosomal abnormalities in a majority of patients are CML (*bcr-abl* fusion in the Philadelphia chromosome resulting from t(9;22) translocations) and APL (translocations of the t(15;17) variety that produce chimeric RARa proteins) [164]. Moreover, even CML cannot be considered to be a single disease, but a heterogenous mix that involves a cluster of various deletions in the *bcr-abl* fusion, some more aggressive than others. Similarly, at least four distinct fusions of RAR-α exist in APL, some being refractory to ATRA therapy but able to respond to treatments that lift the differentiation block at other points in the ATRA-response circuit. All other human adult leukemias present multitudes of abnormalities some-

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times hitting the same oncogenes over and over, sometimes very different genes or gene combinations, with some alterations being shared by a variety of leukemias and chronic myeloproliferative disorders (CMPDs), and some genes being hit by very different malignancies (see **Table 2**). It is possible to find common cytogenetics to the set of myeloid leukemias (see **Table 2**), but various disorders (leukemic and pre-leukemic) typically share one or more genomic mutations; for example, deletion Δ(20)(q11q13) and duplication dup(1q) are shared by AML, PV, ET and myelofibrosis [165]. Other chimeric proteins resulting from *bcr-abl* fusions different from those observed in CML are involved in different leukemic phenotypes, those of ALL and CNL (chronic neutrophilic leukemia, likely just a CML subtype [166]).

The genetic diversity of leukemias has been taken to indicate that the main characteristic of the neoplastic cell is its genomic instability. Our own view is that the diversity merely reveals the recursive nature of the multiplicity of regulatory and signal-transduction circuits that can be used and altered, and which is exposed whenever the cell is forced to experiment with post-adaptive mutations that respond to neoplasia-promoting pressures in the local environment of the cell. Each neoplasia-promoting constraint pushes the target cell to replace the normal tissue regulatory circuit with a bypass circuit, whether it is based on hormone hypersensitivity or independence from hormonal control, and whether the cell employs recursive pathways that may be fetal or embryonic, or instead creates new pathways involving genetic mutations or gross alterations.

Modern theories of cancer have been forced to accept that other genes, besides oncogenes, are involved in the etiology of cancer. Tumor suppressor genes (eg *p53*, *RB*, etc) have become an accepted part of the revised oncogene theory - even though it appears that their role is greatly overrated, and of doubtful *in vivo* correlation with the actual development of neoplasia. One may argue, for example, that, in nearly 3 decades of investigation, the variability of *p53* gene expression and its effects has virtually rendered its function unintelligible. The p53 protein is generally assumed to transduce the response to various cellular stresses - from hypoxia to nutrient deprivation, telomere erosion and defective DNA repair - into tumor-suppressive apoptotic signals, at different stages of the oncogenic process. Yet, targeted mutation of *p53* is seldom, if ever, one of the earliest events in the oncogenic vector. In murine skin carcinomas and human colon carcinomas it is mutated during progression from preneoplastic to neoplastic stages (for a review see [140]). More paradoxically still, loss of the *p53* gene renders mice less susceptible to carcinogen-induced papillomas - not more susceptible. The equivocations lead to argumentative presentations of the role of *p53*, which typically employ such turns of phrase as “one probable reason is”, another intriguing notion is”, “consequences are probably minimal in p53-positive cells” [140], and so forth. To think that it was once dreamt that genetic manipulation of *p53* might one day cure and prevent cancer is, to say the least, sobering. Even the

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RB (retinoblastoma) gene is actually overexpressed in some colon cancers and protects the tumor cells from apoptosis [167], its product acting as both a tumor-suppressor (or apoptosis inducer) and a tumor-enhancer (suppressor of apoptosis and enhancer of proliferation) in the unphosphorylated form. In many cancers, tumor suppressors are neither inhibited or mutated. As an oncologist, one wonders at times whether such terms as "tumor suppressor" are not foisted upon oncology and our understanding more as a function of marketing and capitalizing medical research than as a function of genuine biological discovery or identification of actual functions.

While tumor suppressor genes were limited to act either in support of proliferation (being tumor-enhancing...) or in favor of apoptosis, the identification of proteins and genes that could negatively regulate proliferation without apoptotic effects was of greater interest. The requirement for stimulation with positive growth factor signals was, at every major intersection of every circuit, gated by the antagonist action of growth inhibitory factors. One possible etiology of cancer was that suppression of the expression of negative regulators led to uncontrolled proliferation or hyperplasia [101], at the very initiation of cancer. In this model, the unregulated proliferating cell and its expanding clone was the prime target of initiation. Implicit to it was the notion that differentiation and proliferation were each a composite of positive 'Go' signals with negative 'Stop' signals, the latter being normally provided by expression of negative regulators. At one time, one of the best candidates for a genuine endogenous negative regulator and leukemic growth suppressor [102] was later identified to be Superoxide Dismutase (SOD) [169], raising the prospect that the action was essentially mediated by the presence of molecular oxygen (something that might well have been dismissed too fast as being 'trivial'; see [139]). Other growth inhibitory factors have been proposed, in particular transforming-growth factor-β [170] and the interferons [171], whose action suppresses phosphorylation of the RB protein, as well as c-*myc* expression.

More recently, an alternative proposal regarding the etiology of cancer suggested the existence of 'master genes' that control the distribution and assortment of chromosomes during cell division. Inhibition or inactivation of these genes would be the random cause of the chromosomal instability of cancer cells (the model of Lengauer *et al* [171-172]). However, no such master genes have been found, and this kind of model runs counter to the more recent realizations that biological circuits are regulated by a web of recursive, interconnected and interpenetrating networks of genes, metabolic enzymes, and even including contributions by micro-organisms from various sources or origins. It is as if states of health and disease always translated some form of symbiosis, some form of a collective network between very different elements (a fundamental heterogeneity), with some assemblages of genes, enzymes and micro-organisms being at work in health, and other distinct assemblages in disease processes. Moreover, the "master gene" hypothesis seems not to realize that the directions

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taken by various processes of oncogenesis respond to distinct, tissue-specific and developmental stage-specific selection pressures present in the environment of cells that are at risk of engaging in such processes.

Another theory of how the massive mutations observed in transformed cells may come about as a consequence of gross chromosomal alterations suggests that a mere error during mitosis would suffice to generate aneuploidy (the model of Duesberg at al [173]) and initiate cancer. In our view, this hardly explains anything. Such genetic errors in assortment, etc, can be taken for granted, and again what matters is that only those "errors" (and others) that effectively *work* may be selected by the cell subject to cancer-promoting tissue-environments - or even be brought about and *not* qua errors *per se*, by post-adaptive mutations that favor aneuploid states.

While consideration of the nonviral etiology of most cancers resulted in the enlargement of both the class of oncogenes and the concept of the oncogene, the discovery of the biology of growth factor regulation and the plethora of oncogenesis pathways further expanded them. Thus the concept of the oncogene came to include, as we have said, genes for signal transduction, transcription factors, chromatin remodelling and apoptosis regulation, and very recently the so-called microRNA genes. Signal transducers are either nonreceptor protein kinases (such as tyrosine kinases encoded by the *abl* and *src* genes, and serine and threonine kinases encoded by *raf1* or *mos*) or guanosine-triphosphate-binding proteins (like those encoded by the *ras* gene) that link activated receptors to downstream signal effectors. Transcription factors (such as AP1, which is formed as a dimer of Fos and Jun proteins [174-175]; or the ETS family of regulators present in all metazoa [176-178], some being transcription activators and others transcriptional repressors [179]) can promote or repress expression of genes that participate in the control of cell division, differentiation and apoptosis. They are activated by chromosomal translocations in lymphoid leukemias [152], in sarcomas and prostate carcinomas [180]. Chromatin remodellers are enzymes that alter the position of the histone-composed nucleosomes [181] or modify the N-terminal tails of histones [182-183], and which are subject to epigenetic control. They appear to play a central role in AML and ALL.

The extent to which, under stress-inducing altered physiological conditions, apoptosis regulators play a role in transforming cells - in initiating cancer or controlling its progression - or, alternatively, in committing transformed cells to apoptosis, remains rather unclear. There is a tendency in the apoptosis literature to confuse differentiation and cell death (as if the 'proliferation-STOP' or differentiation-GO' signals were identical to the apoptosis signal), and to welcome anything that might serve as a cancer treatment by the induction of cell death. It is true that differentiation eventually leads either to senescence (see **Fig. 6** top), or to apoptosis, and even true that differentiation is often a slow apoptosis (eg production of outer layer of skin or of hair, or the production of RBCs and their

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progressive degradation by cytoplast loss in the spleen, etc). But a slow apoptosis that is functional as a differentiated state should not be confused with apoptosis or autoschizis resulting from abortive, dysfunctional differentiation, or from tumor killing proteins or factors that may go so far as to include exposure to atmospheric oxygen. Moreover, a proliferation-STOP signal generally introduces a cell into a G1 interphase, whereas the coexistence of a 'proliferation-STOP' signal with a differentiation-GO' signal induces instead a commitment to both terminally proliferate and differentiate.

Upregulation of apoptosis inhibitor genes may be simply an accessory change in many different malignancies. But it has been suggested that the BCL2 family members may be involved in cancer initiation. For instance, *bcl2* gene upregulation appears to be involved in the initiation of almost all follicular lymphomas, some B-cell lymphomas and possibly chronic lymphocytic leukemia (CLL) [184-185], and *bcl2* encodes a protein that localizes to mitochondria and inhibits apoptosis [186-187]. Yet, in all these cases, it is unclear whether initiating events hidden behind and before lymphoma formation might not have originated neoplastic lesions that did not involve *bcl2* upregulation. In the stress pathway of apoptosis, regulatory proteins bind the Bcl2 proteins to inactivate them and trigger the caspases 3, 6 and 7 responsible for cell death [129] (caspases are cascades of cysteine aspartyl proteases that cleave a variety of cellular substrates, literally digesting the cell structures). The stress pathway is a cytochrome *c*-mediated mitochondrial response to cell damage (cytochrome *c* is released from the mitochondria to the cytosol) - whether the damage is mechanical, brought about by deprivation of growth factors or nutrients, or the result of an activation of oncogenes (viral or nonviral in mediation). Conversely, the death receptor pathway (mediated by the CD95 receptor family) is activated by various ligands, including tumor necrosis factor-α, and turns caspase 8 on.

Finally, the conceptualization of microRNA (miRNA) genes as oncogenes creates a new subclass of the latter containing genes that exert regulatory control upon DNA expression *without* encoding a protein. Effectively, these genes only encode short RNA polymers (21 to 23 nucleotides long) that act as negative regulators of the mRNA transcribed from other genes [188], by complementarily annealing to the 3' untranslated region of mRNA to block its translation and cause degradation. Gross chromosomal alterations frequently involve regions rich in miRNA genes [189], and deletions and down-regulation of *miR-15a* and *miR-16-1* genes in most indolent cases of CLL has led to the suggestion that it is an early or initiation event in CLL [129, 190], since it activates *bcl2* expression to prevent apoptosis [191]. Down-regulation of *miRNA* genes in transformed cells seems to occur by a variety of pathways - deletions, epigenetic silencing, and loss of expression of transcription factors. However, in other malignancies, the *miRNA* genes are, on the contrary, overexpressed or up-regulated - even in the same malignancy, as is the case of the *miR155* gene in the more aggressive forms of CLL [192]. The same gene is overexpressed in diffuse large B-cell lymphoma [193], and apparently in a

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variety of breast, lung and colon cancers [129]. Transgenic mice that were transfected with *miR155* under the control of a linked Ig gene enhancer developed ALL and high-grade lymphoma [194], but only after many months. The *miR191* gene is also up-regulated in various tumors [195]. Other microRNAs are downregulated in carcinomas [196]. In general, upregulation of microRNA genes involves either DNA amplification, dysfunctional activation of transcription factors or promoter demethylation [129].

The contrary regulations of the expression of microRNA genes in malignancy has been explained as a circuit where these genes, when overexpressed, function as oncogenes by down-regulating the expression of tumor-suppressor genes, and when silenced, function instead as tumor-suppressor genes that down-regulate oncogenes [129]. Yet, down regulation of *miR-15a* and *miR-16-1* genes causes overexpression of *bcl2* and prevents apoptosis. Thus it would seem that a large variety of microRNA genes are changed epigenetically or genetically in all cancers, whether leukemic or forming solid tumors, and that these changes are all *sui generis*, some resulting in upregulation, others in down-regulation or silencing. Even if they may be prognostic markers of the evolution of specific malignancies, these changes are most likely fine post-transcriptional adjustments that balance positive and negative modulations and are par for the course, rather than initiating events.

Cancer initiation has remained opaque. If anything, it has forced oncology to realize that every successful process of malignant transformation entails cooperating and correlated changes in large arrays of genes, whether these changes are mere alterations in gene expression or adaptive genetic mutations, with the genes involved being not simply oncogenes, "tumor-suppressors", DNA-repair genes, and microRNA genes, but genes controlling all the main functions (energy metabolism, progression through the cell cycle, RNA translation and DNA replication, cell division, commitment to differentiate) and all the essential molecular 'workers' of the metazoic cell - from growth factors and their receptors, to DNA binding proteins, transcription factors, cell surface and nuclear receptors, signal transduction proteins, cell adhesion proteins, transport and carrier proteins, stress response proteins, etc. Initiation itself must involve selection of a multiplicity of experimentations and changes in many of these circuits, since the cell is regulated by so many recursive and multiply-intersecting signaling circuits. Long-gone are the optimistic notions that mutations in two genes (as was once thought from transfection studies with immortalized or established cell lines in serum-contaminated media [52]), or in a few more, might suffice to produce neoplastic transformation. The notion that transformation might involve simple event kinetics has essentially evaporated. Some (Carlo Croce and his group) have suggested that at least most lymphomas and soft-tissue sarcomas are initiated by pathological activation of a single oncogene, followed by changes in other oncogenes and tumor-suppressor genes [129]; and also in parallel, that loss of function of a single tumor-suppressor gene is suf-

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ficient to initiate most carcinomas, before secondary changes comparable to those of lymphomas and sarcomas take place [197-198].

In accordance with the models of sensitivity and independence found for growth factors in transformed hematopoietic cells, it is likely that initiation tends to focus on a particular regulatory pathway most adversely impacted by given oncogenesis-promoting conditions. But even in these cases, it is most probable that initiation will entail a multiplicity of correlated changes, as has been found for PV with the concurrent involvement of (1) IGF-IR-mediated IGF-I hypersensitivity [114, 199], (2) the mutation of the Janus tyrosine kinase *JAK2* gene *that PV shares with all the CMPDs* [199-204], and (3) the overexpression of *SOCS* genes in erythroid progenitor cells - as shown by our more recent work [205]. If the *JAK2* mutation (*JAK2V617F*) shared by all CMPDs is the closest we may come to an initiation event, even this event is not separable from a multiplicity of other correlated events that generate the disorder's phenotype. We should also note that constitutive activation of STAT proteins is frequently found in tumor cells or derived cell-lines, and abnormalities of the JAK/STAT pathway are associated with other types of human cancer (eg breast carcinoma) [206-208]. The involvement of JAK2 in all CMPDs raises the question of what follows in the oncogenic vector after such an initiation. Frequently, the evolution is aggressive and made towards AML, and it is notable that a case of AML has been found to have no other detected cytogenetic abnormality, but a *bcr*-JAK2 fusion gene due to a (9;22)(p24;q11) translocation [209]. But CMPDs may evolve towards CML instead, as found recently in a *JAK2V617F*-positive PV patient who developed a *bcr-abl* translocation that transiently inhibited the PV [210] (note how this also exemplifies precisely the notion that initiating, preneoplastic, hyperproliferative lesions may well lay hidden behind a malignancy that is detected only by its much later stage of progression along an oncogenic vector). Moreover, PCM/JAK2 fusion appears to be a recurrent abnormality in both AML and CML [211]. Even more provocatively, JAK2 translocations have been detected in both pre-B cell ALL and T-cell ALL [212]. It is therefore conceivable that the Janus kinases, the SOCS2 and SOCS3 genes [205], as well as the insulin/IGF-I circuits, may play a pivotal role in hematopoietic malignancies, in both initiation and progression.

The result from all these discoveries is that cancer cell is no longer seen as just an histological anarchist that rebels against the organismic logic and will ultimately engage an amoeboid-becoming. It is a cell that, in response to pathophysiological conditions, grafts by invention entirely different and varied 'micromolecular' shortcuts in the existing regulatory circuits of cells and organisms, so as to achieve independent energy metabolism, growth and proliferation.

Confronted with the myriad of genetic experimentations carried out by cancer cells (more than 100 distinct abnormalities in **Table 2**, which, by the time the present review is published, will

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already be outdated by a few ten more aberrations having been discovered!), one is reminded of what Jack Schultz pointed out back in the late 1950's. Paraphrasing him - that (1) *since* no one aberrant chromosome number or set of chromosomal or genetic changes is characteristic of a malignant cell; and (2) *since* so many aberrant chromosome types are found in malignant cell populations with very different phenotypes; and (3) *despite* the increasingly apparent nonrandom nature of most of these aberrations, one is forced to conclude that either each malignant phenotype is controlled by many, alternative, recursive and interacting genetic loci, or malignant transformation is insensitive or indifferent to most chromosomal alterations present in cancer cells (they are 'fluff' or 'ornamental').

Today, more and more so, the answer appears to be the first arm of the alternative: there are many recursive genetic loci and regulatory circuits in a cell, and it is always a multiplicity of such loci or such circuits that is affected in each cancer cell - or each clone of cancer cells. But, more profoundly still, the answer appears to be that the cancer cell aims at sustaining a state of genetic experimentation which translates into an apparent chromosomal instability or 'creativity'. It does not need a virus, or any other element, but the ordinary cytological elements that it already has, in order to undergo a process of initiation or begin to transform. It is the tissue cell itself that commits to transform, in what is best described as auto-oncogenesis - the transformation of a cell by itself, or on its own, in response to an adaptive pressure. That the oncogenic vector targets an organ or tissue, and that it recurrently attacks it with many tissue cells switching on auto-oncogenesis simultaneously and at different times, underlines how cancer-promoting pressures have both a systemic nature and a localized impact. Since tumors are typically heterogenous (polyclonal) in their cellular composition, one can think of them as an ongoing biological reactor where all cells are experimenting with the adaptive power of complex epigenetic regulations and genetic alterations. Most tumor cells fail in their experimentation to become neoplastically transformed - in their quest to adapt ever more successfully to hypoxic and dysfunctional environments, so we suggest [139]. In fact, this is not just true of malignantly transformed tumor cells, but just as well true of leukemic cells, or transformed leukoblasts and erythroblasts. A study of cell death in Friend erythroleukemia showed that hyperplastic Friend spleen cells daily underwent a massive cell death (84% of splenic DNA had a daily turnover) and were not malignant (or successfully transformed) [74]. Autonomous genetic experimentation is ongoing at every stage of oncogenesis, in hyperplastic and in neoplastic stages, in initiation and in progression, and will select for those genotypes that adapt to what we suggest are intensifying hypoxic and free-radical stresses that impel the auto-oncogenic process forward [139]. In leukemias, diverse transformed clones will coexist in the blood system, until a faster-growing and metabolizing transformed clone takes over the sites of hematopoiesis, which may be extramedullary. For example, evolution of PV towards AML is not a progression that occurs within the PV clone (thus, the continuity of the oncogenic vector can

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only be addressed as a function of persistent and consistent cancer-promoting pressures in the tissue environment). Even though the origin of tumors is clonal - as first established by P. Fialkow in 1974 with X-linked markers [214] - the growth of tumors results in clonal heterogeneity, the diverse transformed clones symbiotically interacting inside the same tumor [215], until, when and if, the clone of a more aggressive neoplastic genotype takes over the tumor, or is able to metastacize beyond the tumor, become tissue-invasive and seed its own colonies in new locations.

It is the richness and the variety of both the adaptive and epigenetic experimentation by cancer cells that precludes all persisting tendencies to reduce its etiology to the random accumulation of a few well-defined (pre-adaptive) mutations - virally caused or caused by chemical and physical insults - that may be passed vertically, in a minority of the cases, to give rise to familial or hereditary forms of cancer. The variety of recursive regulatory pathways that can be hit at initiation of oncogenesis and during its progression is most likely the result of the development of a "multitude of protective mechanisms" designed to control proliferation and DNA mutation in organisms with increased complexity [215]. What we call the errors in these "mechanisms" may well turn out to be the result of a strategy on the part of cells and organisms to leave open the possibility of generating directed mutations productive of adaptive changes. It is this strategic disposition which the cancer cells hijack. A decade ago, John Cairns and others drew precisely attention to this [215]. The evidence ("from epidemiology, experimental carcinogenesis and molecular biology" [215]), as we stated above, suggests that a fully transformed cancer cell requires mutation of a large number of genes. Assuming the human body has an estimated 10^{10} target stem cells, even if each one of them divides once every other day (in mice they do it once a day [215]), an individual who will live 70 years will only have gone through 10^{14} stem cell divisions. Assuming oncogenesis to be a neo-darwinian process of random pre-adaptive mutations with the accepted rate of 10^{-7} mutations per gene per cell division, "it is hard to see how any of these cells can acquire enough mutations to become cancerous, unless some process is raising the mutation rate far above its usual value" [215]. The product of two mutations in different genes would exhaust the entirety of the stem cell divisions in the lifetime of a human being. Yet, 1 in 3 human beings presently contract cancer, with an annual rate of 10 million per year (the rate increasing with advancing age), and with every human organism thwarting survival of many, many transformed cells during a lifetime. Thus Cairns suggested that "we should be looking at some other driving force that can be linked to (or triggered by) cell proliferation" in trying to explain the rate of cancer production. This clearly made the case for post-adaptive genetic changes, as well as drew attention to the strategy implicit in the wild proliferation of cells engaged in oncogenesis - the abnormal expansion of stem cell clones increases the chances of the cells experimenting with a more extensive array of transforming mutations, specially if these mutations hit genes involved in DNA repair or in the control of

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mutation rates (as *p53* was once thought to do, when it was called 'the guardian of the genome').

Now that we at last have begun to learn from understanding how growth factors and the intracellular signaling associated with their action control and modulate metabolism, growth, DNA replication, cell division and differentiation, we are left with *no single* biological explanation for all cancers, nor with any understanding of whether there is a unifying factor - or neoplasia-promoting pressure - universally required for the manifestation of cancer. Undoubtedly, as most cancers are functionally acquired, they are induced by the environment, whether the environment is external (case of physical and chemical mutagens) or internal (case, for example, of hypoxia, or of starvation) to an organism or living system. The function of external factors in the induction of malignancy is today well demonstrated by epidemiological studies. But aside from cancer induction by "ionizing radiation" (including in this term free-radical inducing UV-B and UB-C radiations) and a few chemical carcinogens, experimental carcinogenesis with most compounds, viruses, mycoplasma and bacteria (eg *Helicobacter pylorii*) did not succeed. Tobacco smoke is not carcinogenic in most experimental animals [215], and in studies where it appears to be carcinogenic, the effect of tobacco proper was never separated, for example, from that of the burning of paper containing heavy metal salts; very few viruses induce human malignancies and seemingly cannot do so by themselves; ditto for mycoplasma [135], and *Helicobacter* is not even mutagenic. Induction of melanoma by UV-B or UV-C is far from being a straight causal relation, being proven only in patients that have germline defects in nucleotide excision repair [216]. Thus, modern oncology ended up discussing the risks of 'this or that cancer' as a function of accumulated injuries by a variety of mutagenic factors, rather than talk about the causes or the actual induction of cancer. What has become clear is that cancer is a disease of deregulated cell proliferation, and thus that the initiation event must bear upon a proliferating cell and aim at sustaining its state of proliferation. Apparently, transformed cells can do so in a great many ways.

Is there then no unifying understanding of cancer because no such understanding is possible? Because - even though there is an oncogenic vector, or there are consistent oncogenic vectors - there is no single process of oncogenesis, no unifying factor in cancer causation? Because cancer is just a name for a very large variety of diseases? Or is it that, for too long, molecular biology has concentrated on the mechanisms of cellular signaling, genetic susceptibility, genetic alterations and DNA expression, post-transcriptional control, etc, and disregarded the role of dynamic and economic factors in the understanding of cancer? Could oncology be missing what is of essence in the commonality of all cancers? If, to follow Temin, the retroviruses and other oncogenic viruses are, or can be, *de novo* (effectively heterogenic as we said above) neo-lamarckian creations from a cell experimenting with its responses to an adversely changing environment, so can the transformed cell genotypes that result from such experimentation be the result of directed, neo-lamarckian mutations by the cell itself,

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in fulfillment of a commitment to transform. And it may well be that these neo-lamarckian post-adaptive mutations, despite the myriad of possible combinations, have a unitarian biochemical and biophysical basis - as we have punctually suggested above may well be the case with hypoxia and malnutrition (in particular, dietary lack of essential factors, such as vitamins A, D, and C) [139]. Thus, in a completely auto-oncogenic model of cancer, the unifying condition may turn out to be as simple as lack of readily available energy to the cells, that is, lack of the right kind and quantity of energy required by the living system to complete the normal programs of tissue function, expansion, regeneration and differentiation.

10. A note of caution regarding mainstream science and mass-media sensationalism

When viral oncology was young, it met with much derision from classical geneticists and the media of the time. But then the quantitative methods introduced by Max Delbrück into microbiology, their extension to tissue-culture, the advances in transmitted electron microscopy, the discovery of DNA, the bacteriophage work of Lwoff and his group at the Pasteur Institute, the retroviral induction and transmission of murine leukemias by Gross in New York, etc, turned the tide in the medical and research establishment of official science, and in the media and public opinion, in both the US and Canada. Oncology had found a new approach and the Canadian Cancer Society (CCS) and the NIH were at the forefront of new research. In Canada, it started with "the virus committee" of the CCS headed by the great Arthur Ham, and composed of Arthur Axelrad, Allan Howatson, E.A. McCulloch and Louis Siminovitch. The committee seeded the Ontario Cancer Institute where some of the most advanced research in induced viral oncogenesis was conducted. It put forth the notion that the rampant cell division of the cancer cell was the result of an attack by a virus, whether acquired infectiously or vertically [217]. These investigators and others like them in the US and France at the time were all intelligent scientists who had also learned how the media wields power and how its publicitary structure can be used to raise capital for medical and basic research. From the beginning, and no matter how basic was the research, it had to be linked - by the scientific and medical establishment, by the media and in the mind of the public - to the benefits it could bring to the potential cure of cancer. To investigate whether cancer was caused by viruses was not separable from the hope of finding a vaccine that might cure cancer. Some scientists did not hesitate to declare that "cancer is curable now, in its early stages" [218].

Eventually, all cancer research institutes and hospitals, and all cancer societies came to rely on this constant media marketing of hope in order to launch funding drives tightly coordinated with the reporting of research news. Cancer research became increasingly more vetted by media reporting than by genuine peer-review, the latter more and more plying to the former, or to current fads tied to the

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marketing of pharmaceutical "anticancer drugs", and the same criteria came to rule public and private granting agencies. Though the apparent intention was to finance basic research, and thus laudable as such, over time the funding inevitably flowed to research that could be sheltered under the promise of a cure - leaving the clinicians either with pipe-dreams (anti-cancer vaccine, cancer genes that could be shut down, interferon cures, GH prevention, the cure-all stem cell, etc) or with treatments of, at best, doubtful value and which were onco-iatrogenic in turn (radiation therapy, chemotherapy). Aside from legal regulation of exposure and safety in industries where the cancer risk is obviously high (nuclear power plants, dry cleaning, the asbestos industry, etc), little else was of benefit to society or to the cure of cancer. This was so, even if, despite the dominant research directions, many of the research efforts produced a wealth of epochal findings in basic science - from viral oncogenesis to the biology of growth factors and microRNA genes. But for basic science, even worse would come to pass, as growing hospital and academic bureaucracies absorbed the greater part of the public funding of medical and scientific research. The cancer funding drives had become a way of existence, an industry on its own, and its power could be felt politically and socially through the emerging technobureaucracies. Thus, on the basis of perfectly unsound research regarding nicotine addiction carried out and suppressed by some of the tobacco company giants, the political power of the tobacco industry was cut down to size by other corporate and political lobbies in the US federal government. Under the rubric of carcinogenesis induced by mutagens, smokers were legally and politically transformed into a minority of second class citizens by federalizing bureaucracies that vastly increased their power in the act (such as the Office of the Surgeon General in the US, or the NIH, CCS, etc).

The inability of clinical and experimental oncology research to come up with a unifying theory of the etiology of cancer, together with the ravages that cancer has increasingly wrought in association with the so-called processes of industrialization and modernization, created a fertile market for the constant faddist speculation on the causes and cures of cancer. It is to this fadist and populist market that modern medical and scientific research must now ply. This, at least as much as the complexity of cancer itself, has been a detriment to its real, functional understanding.

At the apogee of the viral oncogene theory (late 1980's and early 1990's), the mainstream media marketed it with the approval of the organs of official science (academias, professional societies and journals), until the famous viral oncogenes were found to be transduced from cellular oncogenes present in all cells, and which could be altered with no overt or covert signs of viral involvement. But in between and in the aftermath, the mass-media had meanwhile found greater risk of cancer for beer and wine drinkers; believed that the causation of cancer by cigarette smoking was a proven fact - a dogma to this day; claimed that estrogen could cause mammary cancer but recommended usage of the pill to prevent ovarian cancer; then unabashedly claimed the pill increased malignant melanoma;

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linked coffee and decaffeinated coffee to liver cancer; claimed chlorinated water as cause of increase in cancer; claimed bacteria in semen were cause of cervical cancer [219], then that it was HPV that caused it; next that talcum caused ovarian cancer; that saturated fats caused cancer; etc, etc, in a merry go round which was duplicated with even more absurd claims in the marginal and alternative media. For, indeed, what has marked the alternative media in this regard is not a more loyal adherence to science, methodology, verification, thought or caution; no, what has been its dominant marker since the days of counter-culture is adherence to the fantastic, the hopeful, the miraculous. In fact, at the limit, the distinction between official and alternative oncology has today blurred. Frequently, the alternative milieu keeps alive as myths what were once theories or dogmas in established oncology long past their demise. Other times, it is the so-called forefront research that itself is mythical.

Consider the social and scientific derangements entailed by the discovery of cellular oncogenes, the expansion of their concept and type, the complexity of their control and mutagenesis, and how chromosomal aberrations typically target them. As Cairns frequently pointed out - to know all this is still not knowing how a cell transforms or initiates a cancer process, piece by piece. For we are simply reading code alterations that were selected by a biological process, and not alterations that guided the process or initiated it - as randomistic views of cancer causation still hold is the case. Yet, no matter how diverse are these genetic abnormalities, and despite the fact that we do not even know how many of them there are effectively involved in oncogenesis and neoplasia (there could be a million variations, etc), there are plenty of medical geneticists and oncologists who believe it is possible to quantify the cancer risks associated with every *known* genetic locus or marker, and by a variety of fuzzy statistics, to quantitate a probability of a specific cancer for someone whose genome has been sequenced or scanned for markers. Apart from the selling of such genomic tests being a veritable snake vendors' paradise, this myth - or mythological belief - is a fabulous tool for all agencies engaged in control and modification of social behavior, and constitutes a mine yet to be explored by insurance companies: the equivalent of what the carbon market economy did for the pseudoscientific myth of global warming. As usual, the first consumer of this hi-tech so-called "prophylaxis of cancer" was the community of Hollywood stars in Beverley Hills. But the world and the internet today are sprinkled with clinics that will scan anyone for 'computable' genetic risks of all sorts of diseases, not just cancer. There are cases we know of healthy women typing positive for *nonfamilial* markers of cancer of the breast or uterus, etc, who voluntarily underwent medical castration just to be able to sleep at night. One wonders if addiction to bad genetics, to a poor understanding of how genetic encodings really work, is also an iatrogenic derangement or, more properly speaking, a derangement inherent to *a* science that likes to appear to control what in effect does not - just as, in the past, parallel derangements occurred, *viz*. how classical genetics went from being Mendelian to Social-Darwinism, Nazi

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eugenics and Lysenkoism. Perhaps in the recent past, to establish an Oedipus complex a content to the complex was still required, with castration simply blocking the emotions sourced in the repressed organ. Today, there is hardly a need for the complex when self-castration of targeted organs can be induced as a prophylaxis on the basis of, at best, very dubious genetic probabilities. It was only a decade ago that the older notion of male surgeons that a hysterectomy relieved a woman of a 'cancer trap' was shown to be a myth without causative or statistical foundation. Now the myth has returned, but it is genetic probability that dictates the castration. It is no longer a male prejudice.

Thus, it is hard to ascertain who are the parties responsible for the ingrained mythology that surrounds the causes and cures of cancer in the public mind - whether it is the scientists who lend themselves to these circuses with the excuse of needing grants and the desire for the limelight; whether journalists and media-makers are simply marketeers, or pimps and impotent, too, at investigating anything; or whether the public is gullible out of ignorance or a need to be entertained, even when confronted with the most ravaging of diseases. All this, even though a substantial number of these scientists, journalists, media-makers and media-watchers will develop cancer and likely die from it.

What is certain is that, just as yesterday the media was the enemy of medical oncology and ridiculed all novel theories and findings - not just Reich's orgonomic approach [7], but just as well Lwoff's bacteriophages or Gross's experiments in viral oncogenesis - the world of the media and instant electronic communication is also today the enemy of oncology research and the cancer patient. But the "how" is now inverted. It is not lack of coverage, malevolent gossip or exaggerated and irrational skepticism. Rather, it is sensationalism, it is the irrationalism of ready-made beliefs taken to the maximum. This is what the same old packaging of hope by the medical institutions and their scientists and the marketing of alternative treatments and publications now have in common, that belief can be micro-manufactured and micro-managed, that it can be packaged just as well in the latest discovery of institutional science, as on word of a conspiracy to suppress a miracle cure by doctors or the establishment against 'the rest of us'.

Those with the most sense for business talk of many cancer cures - of a panoply of antibodies engineered against all the receptors, or signal effectors, etc, that may be activated in a variety of cancers, as if the vision of a single vaccine had decayed into the vision of a million vaccines. Thus, like any other social establishment, oncology has become subject to these recurring waves of fads, each recurrence bringing a new twist. With each twist, a glorious cure is promised. A recent effusion penned by the Dean of the University of Buckingham Medical School reduced cancer to an entropy of transmission or corruption of cellular information [220]. We imagine all cancers must laugh together at such temerity. For, in fact, the cancer cell should rather be construed as an innovative operator of biological and genetic changes, with the information being, not corrupted, but intercepted,

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deviated, experimented with, modified for specific adaptive purposes of making the cell independent from the organism. Undaunted by the variety of cancers, and with the firm belief that engineered molecules will be able to treat each cancer, the Dean dreams not of a glorious cure, but glorious cures: a myriad of "right medicines", each to be given "to the right patient at the right time" in tourist-like resorts, all the result, he says, of "consumerism increasing in medicine". He freely anticipates cancer in all its variants to become controlled by 2025. We think it must be another Nostradamus prediction.

In light of such mediatic exhibitionism by medical scientists and clinicians having become ordinary occurrences, what then has become of the difference between established science and pseudo-science - between modern oncology and its clinical practice *and* pseudo-oncology with its wanton experiments, volunteer fake treatments and "wikipedian" results?

The caution, then, is to a society that permits such a degradation of basic and clinical science that, at the limit, turns oncology into mythology and fuzzy logic, and myths become entitled to acquire a scientific status. Such a society has lost sight of the importance of basic research, and of the fact that basic research should not be subordinated to social, political and economic interests. As it loses the power to question and investigate freely "the nature of things", it is left only with the power to empower all possible fictions, and give them a rhythm of succession in the world of mass-entertainment. And in that world of fiction, the war against cancer appears to have already been won. The irony could not be more bitter to all who suffer from a disease which can be called the scourge of modern man. It makes a mockery of medical science and deprives medical research of the public support it needs to complete its task of one day understanding cancer in all of its variations so that it can devise non-cytotoxic treatments. The marketing of hope is no substitute for understanding, without which there is no chance for effective treatment, let alone a cure, if there is one.

Table 2 (page on the right and following pages) - Gross chromosomal alterations and genetic mutations so far identified in transformed cells from solid tumor cancers, leukemias and blood proliferative disorders.

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Table 2

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Target gene or fusion genes	Rearrangements	Disorder(s)	Function Involved
1. Lymphomas & lymphoid leukemias **1.1 B-cell: oncogenes juxtaposed by translocation with immunoglobin loci: transcriptional activation**			
1. *c-myc*	t (8; 14) (q24; q32)	Burkitt lymphoma	HLH domain (IgH)
2. *c-myc*	t (2; 8) (p12; q24)	B-ALL	(Igκ)
3. *c-myc*	t (8; 22) (q24; q11)	B-ALL	(Igλ)
4. *c-bcl1 (cyclin D1)*	t (11; 14) (q13; q32)	B-CLL	PRADI-*Cyclin D1*
5.*bcl2*	t (14; 18) (q32; q21)	Follicular lymphoma	Mitochondrial IM protein (apoptosis)
6. *bcl3*	t (14; 19) (q32; q13.1)	B-CLL	CDC-10
7. *IL-3*	t (5; 14) (q31; q32)	Pre-B-ALL (early-B ALL)	GF (IgH)
8. *ID4*	t (6; 14) (p22; q32)	Pre-B-ALL	ID4 overexpression
1.2 Pre-B-cell: gene fusion by translocation			
1. *MLL/AF4*	t (4; 11) (q21; q23)	Pre-pre-B-ALL (M+)	...
2. *EZA/MLF*	t (17; 19) (q22; p13)	Pre-pre-B-ALL	...
3. *bcr/abl*	t (9; 22) (q34; q11)	Early pre-B-ALL	oncogene fusion
4. *PBX1/EZA*	t (1; 19) (q23; p13.3)	Pre-B ALL	HLH (*EZA*) regulated by p300 & core-binding protein (CBP)
5. *TEL/AML1*	t (12; 21) (p13; q22)	Early pre-B-ALL (M-)	...
6. *TEL/JAK2*	t (9; 12) (p24; p13)	Pre-B-ALL	constitutive *JAK/STAT* signalling
7. *SSBP2/JAK2*	t (5; 9) (q14; p24.1)	Pre-B-ALL	...
1.3 T-cell: oncogenes juxtaposed with TRC loci: transcriptional activation by translocation or inversion			
1. *c-myc*	t (8; 14) (q24; q11)	T-cell-ALL	HLH domain
2. *LYLA*	t (7; 19) (q35; p13)	T-cell-ALL	HLH domain
3. *TAL1/SCL/TAL5*	t (1; 14) (q32; q11)	T-cell-ALL	HLH domain
4. *TAL2*	t (7; 9) (q35; q34)	T-cell-ALL	HLH domain
5. *RBTN1*	t (11; 14) (p15; q11)	T-cell-ALL	LIM domain
6. *RBTN2/TCR* α-δ	t (11; 14) (p13; q11)	T-cell-ALL	LIM domain
7. *RBTN2/TCR* β	t (7; 11) (q35; p13)	T-cell-ALL	LIM domain
8. *MLL/AFX1*	t (X; 11) (q13; p23)	T-cell-ALL	...

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Table 2 cont.

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Target gene or fusion genes	Rearrangements	Disorder(s)	Function Involved
1.3 T-cell: oncogenes juxtaposed with TRC loci: transcriptional activation by translocation or inversion cont.			
9. *HOX11/TCR* α- δ	t (10; 14) (q24; q11)	T-cell-ALL	Homeodomain
10. *HOX11/TCR* β	t (7; 10) (q35; q24)	T-cell-ALL	Homeodomain
11. *TAN1*	t (7; 9) (q34; q34.3)	T-cell-ALL	Notch homologue
12. *TCL1/TCR* α- δ	t (14; 14) (q11; q32.1)	T-cell-APLL (T-CLL)	Oncogene activation
13. *TCL1/TCR* β	t (7; 14) (q35; q32.1)	T-cell-APLL (T-CLL)	Oncogene activation
14. *?*	t (2; 11) (q11.2; q15.1)	T-cell-ALL	?
15. *?*	t (14; 14) (q11; q32)	T-cell-ALL	IgH
16. *?*	inv (14) (q11; q32.1)	T-cell-APLL (T-CLL)	?
1.4 Lymphomas (hematopoietic tumors)			
1. *REL*	ins (2; 12) (p11.2 - 14)	T-cell-ALL	NF- κB family
2. Myeloid leukemias and CMPD's **2.1 Mutations, deletions, duplications and supernumerary chromosomes**			
1. *JAK2 (V617F)*	9 p24	PV, CMPD's	IGF-1 hypersensitivity & STAT Activation
2. *?*	del (5) (q13; q33)	MDS, AML	?
3. *?*	del (7) (q22; q34)	MDS, AML	?
4. *?*	del (11) (q23)	MDS, AML-M5, few ALL	MLL deletion
5. *?*	del (12) (p12)	MDS, AML	?
6. *?*	del (17) (p11-12)	MDS, AML	?
7. *?*	del (20) (q11; q13)	PV,ET, MDS, AML	?
8. *?*	dup (1q)	PV,ET, MDS, AML	?
9. *?*	+4	AML-MO	?
10. *?*	+6	MDS, AML	?
11. *?*	+8	PV, AML	?
12. *?*	+11	AML	MLL duplication
13. *?*	+13	AML	?
14. *?*	+19	MDS, AML	?
15. *?*	+21	MDS, AML	?

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Table 2 cont.

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Target gene or fusion genes	Rearrangements	Disorder(s)	Function Involved
2.2 Gene fusion by translocation or inversion			
1. *ETO/AML1*	t (8; 21) (q22; q22)	AML-MO, M1 & M2	CD19+, CD56+
2. *MTG16/AML1*	t (16; 21) (p11; q22)	MDS, AML-M2, M4 & M7; CML	...
3. *NPM/RAR*α	t (5; 17) (p23; q11 - 12)	APL = AML-M3	inactiavates ATRA action on RAR nuclear receptor
4. *PML/RAR*α	t (15;17) (q22; q21)	APL = AML-M3	inactiavates ATRA action on RAR nuclear receptor
5. *PLZF/RARa*	t (11;17) (q23; q21)	APL = AML-M3	inactiavates ATRA action on RAR nuclear receptor
6. *NUMA/RARa*	t (11;17) (q13; q21)	APL = AML-M3	inactiavates ATRA action on RAR nuclear receptor
7. *CBF*β*/MYH11*	t (16;16) (p13; q22)	AML eo	...
8. *DEK/CAN*	t (6; 9) (p23; q34)	AML baso	no homology
9. *AF1q/MLL*	t (1; 11) (q21; q23)	AML - M5 = AMOL	Monocytic leukemia; chromatin modifier MLL
10. *AF6/MLL*	t (6; 11) (q27; q23)	AML - M4 = AMML & AML - M5 = AMOL	Myelomonocytic & monocytic leukemias chromatin modifier MLL
11. *AF9/MLL*	t (9; 11) (p22; q23)	AML - M5 = AMOL	Monocytic leukemia chromatin modifier MLL
12. *AF10/MLL*	t (10; 11) (p13; q23)	AML - M4 = AMML & AML - M5 = AMOL	Myelomonocytic & monocytic leukemias chromatin modifier MLL
13. *MLL/AF17*	t (11; 17) (q23; q21)	AML - M4 = AMML & AML - M5 = AMOL	Myelomonocytic & monocytic leukemias chromatin modifier MLL
14. *MLL/MEN/ELL*	t (11; 19) (q23; p13.1)	AML	Chromatin modifier MLL
15. *MLL/ENL*	t (11; 19) (q23; p13.3)	AML, ALL	Myelomonocytic & lymphocytic leukemias chromatin modifier MLL
16. *Ribophorin1/EV11*	t (3; 3) (q21; q26)	AML Meg = AMeg, MDS	Acute megakaryocytic leukemia
17. *MOZ/CBP*	t (8; 16) (p11; p13)	AML M4 = AMML & AML Meg with erythrophagocytosis	CBP & p300 leukemias
18. *MLL/CBP*	t (11; 16) (q23; p13)	MDS, treatment induced AML	CBP & p300 leukemias

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Table 2 cont.

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Target gene or fusion genes	Rearrangements	Disorder(s)	Function Involved
2.2 Gene fusion by translocation or inversion cont.			
19. *MLL/p300*	t (11; 22) (q23; p13)	AML	CBP & p300 leukemias
20. *MLF1/NPM*	t (3; 5) (q21; q31)	AML-M6 (erythroleukemia)	...
21. H*LF1/NPM*	t (3; 5) (q25; q34)	AML, MDS	...
22. *abl/bcr*	t (9; 22) (q34; q11)	CML, treatment induced AML	tyrosine kinase activated by *bcr*
23. *EVI1/MDS1/AML1*	t (3; 21) (q26; q22)	CML-BC (Blast Crisis), MDS, treatment-induced AML	...
24. *PDGFR*β*/TEL*	t (5; 12) (q33; p13)	CMML	PDGF-receptor, gf
25. *FGFR1/CEP110*	t (8; 9) (p12; q33)	AML, CMPDs	FGF-receptor, gf
26. *?*	t (2; 11) (q37; q23)	MDS induced by treatment	...
27. *?*	t (2; 11) (p21; q23)	AML, MDS	...
28. *JAK2/bcr*	t (9; 22) (p24; q11)	AML	*JAK2* activation by *bcr*
29. *?*	t (14; 22) (q32; q11)	CML	involves *bcr*
*30. CF*β*/MYH11*	inv (16) (p13; q22)	AML eo	CBF & MYH
*31. CF*β*/MYH11*	inv (3) (q21; q26)	AML - AMegL	?
3. Solid tumor-forming somatic cancers **3.1 Gene fusions in sarcomas by translocation**			
1. FL*I1/EWS*	t (11; 22) (q24; q12)	Ewing's sarcoma	Ets transcription factors
2. *ERG/EWS*	t (21; 22) (q22; q12)	Ewing's sarcoma	Ets transcription factors
3. *ATV1/EWS*	t (7; 22) (q22; q12)	Ewing's sarcoma	Ets transcription factors
4. *ATF1/EWS*	t (12; 22) (q13; q12)	Soft tissue sarcoma	Ets transcription factors
5. *CHN/EWS*	t (9; 22) (q22; q12)	Chondrosarcoma	Steroid receptor
6. *WT1/EWS*	t (11; 22) (p13; q12)	Wilm's tumor	Wilm's tumor gene
7. *SSX1, SSX2/SYT*	t (X; 18) (p11.2; q11.2)	Synovial sarcoma	HLH domain
8. *PAX3/FKHR*	t (2; 13) (q37; q14)	Alveolar sarcoma	Homeobox homolog
9. *PAX7/FKHR*	t (1; 13) (q36; q14)	Rhabdomyosarcoma	Homeobox homolog
10. *CHOP/TLS*	t (12; 16) (q13; p11)	Liposarcoma	Transcription factor activation
11. *HMG2-C/?*	t (12; 14) (q13; q15)	Leiomyomas	HMG DNA-binding protein

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Table 2 cont.
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Target gene or fusion genes	Rearrangements	Disorder(s)	Function Involved
3.2 Gene fusions in carcinomas by translocation, inversion or gene rearrangement			
1. *RET/ptc 1*	inv (10) (q11.2; q2.1)	Papillary thryroid carcinomas	Tyrosine kinase activation
2. *RET/ptc 2*	t (1; 17) (q11.2; q23)	Papillary thryroid carcinomas	Tyrosine kinase activation
3. *RET/ptc 3*	inv (10) (q11.2)	Papillary thryroid carcinomas	Tyrosine kinase activation
4. *TRK*	inv (1) (q31; q22-23)	Papillary thryroid carcinomas	Tyrosine kinase activation
5. *TRK-T1(T2)*	inv (1) (q31; q25)	Papillary thryroid carcinomas	Tyrosine kinase activation
6. *TRK-T3*	t (1; 3) (q31; ?)	Papillary thryroid carcinomas	Tyrosine kinase activation
7. *TMPR552*	rearrangement 21q22	Prostate carcinoma	Ets transcription factor activation
8. *ERG*	rearrangement 21q22.3	Prostate carcinoma	Ets transcription factor activation
9. *ETV*	rearrangement 7p21.2	Prostate carcinoma	Ets transcription factor activation
10. *Cyclin D1*	inv (11) (p15; q13)	Parathyroid adenocarcinoma	PRADI/Cyclin D1

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