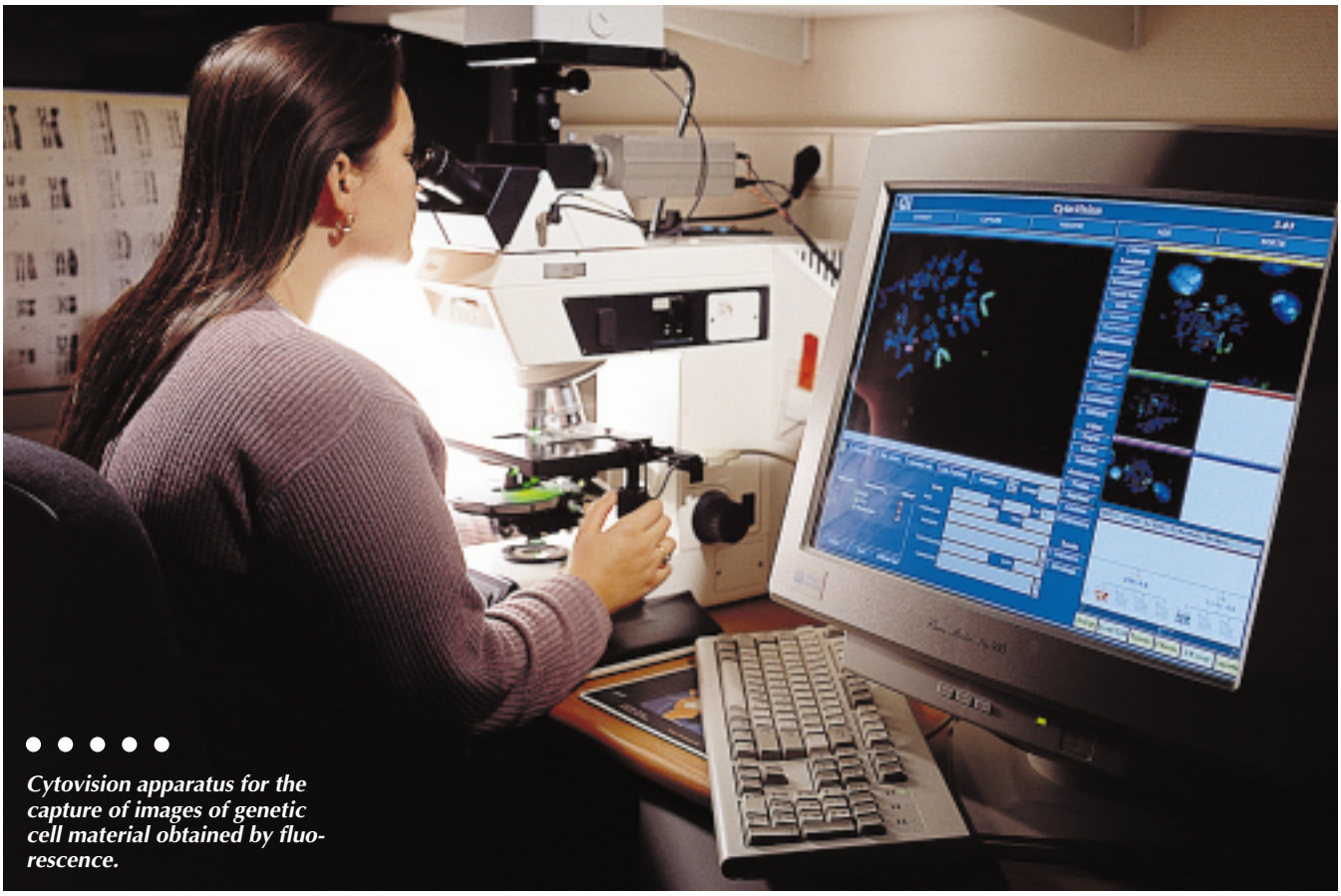
CHROMOSOMAL INSTABILITY

Chromosomal instability, identified by the presence of anomalies in the shape and number of a cell's chromosomes, is observed both during the aging process and in carcinogenesis, as well as after stress such as irradiation. This mechanism seems to be necessary in all cases for the cell to recover its stability, which enables it to pursue normal growth, with a limited life span, or become immortal, as in cancer. Aging is a major source of accumulated mutations and therefore carries with it a greater probability of cancer formation. Even so, a single mutation is not sufficient to transform a healthy cell into a cancerous one: cancer formation requires the coincidence of several rare and independent events.



Cytovision apparatus for the capture of images of genetic cell material obtained by fluorescence.

A. Gonin/CEA

Diverse circumstances

Chromosomal instability can occur both ***in vivo***, during **carcinogenesis**, and ***in vitro***, in the course of **cell** aging, *cell transformation*(1) or after irradiation or exposure to various toxic agents. This characteristic reflects the presence in the **karyotype** of chromosomal anomalies, such as chromosomes with two **centromeres** (**dicentric**), exchange of material between two chromosomes (**translocations**), or gain or loss of chromosomes. These anomalies occur from one generation to the next and affect different chromosomes.

Found in most tumor cell **lines**, **telomeric** associations (between chromosome ends) are one feature of chromosomal instability.

They can be detected in the cells of individuals predisposed to cancer and in cancer cells at their early stages. These telo-

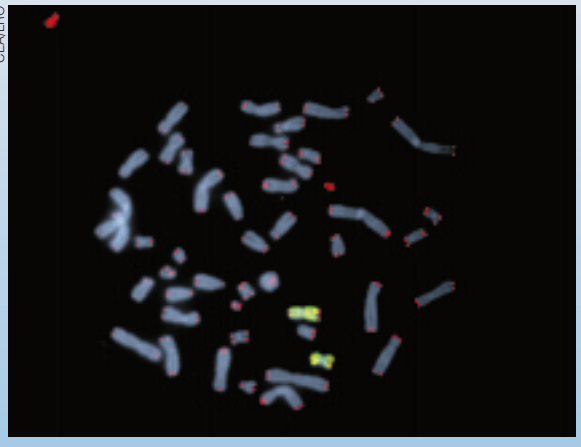
(1) Conversion of a normal cell to an immortal and then cancerous cell.

Telomeres and telomerase: why the ends are important

1

The telomeres at the chromosome ends protect these from **nucleotide** degradation and end-to-end fusion. As early as 1938 research had shown that telomeres had a protective role (capping), ensuring the **mitotic** stability of the chromosomes: after irradiation by X rays chromosomes devoid of them became «sticky» and could initiate break and fusion cycles. The telomeres, which shorten after each cell division, control the proliferative capacity of some of our cells. **Proteins** specific to the telomeric **sequences** form the telosome. Non-recognition by the proteins of the telosome would be enough to transform a telomere into a double-strand break. Sophisticated systems constantly monitor the integrity of the **DNA**. **Repair enzymes** are mobilized to repair any breaks that appear. But this machinery could easily confuse chromosome ends with a DNA break that needed repair. Telomeres might thus get fused together by recombination or by end-to-end fusion mechanisms, which would result in the formation of unstable dicentric chromosomes. The telomeric associations are the starting point of dicentric chromosomes, which in turn generate chromosomal instability (box 1).

CEA/LRO



Cell in metaphase: its chromosomes are colored blue, telomeric sequences red, and chromosome 16 yellow-green.

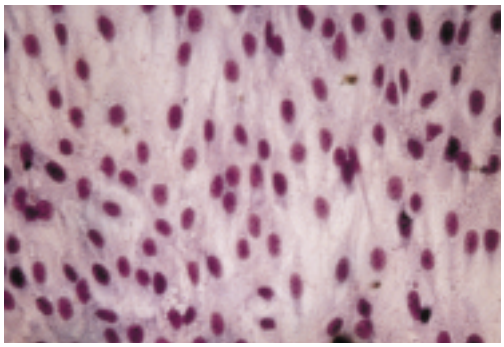
Chromosomal instability during aging

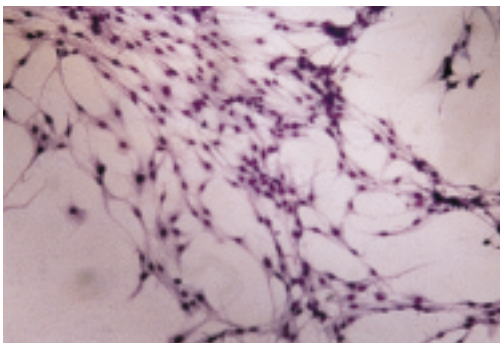
It was accepted in the early sixties that cell aging could be explained by the existence of an upper limit to the number of possible cell divisions. After a set number of generations the cells senesce and die. The aging or **senescent** cells display a marked increase in the number of chromosomal anomalies; the formation of these aberrations generates breaks, which if not repaired cause the **cell division cycle** to stop: after a limited number of divisions these cells will then no longer divide (see *Effects of radiation on the cell division cycle*).

Scientists have shown that primary cultures of **fibroblasts** (cells of conjunctive tissue) live for only a limited number of cell generations, corresponding to about fifty doublings of their popu-

One enzyme, **telomerase**, can lengthen the telomeres. Whereas this enzyme is absent from most **somatic** cells, nearly 85% of tumors show a telomerase activity. Accordingly this enzyme has become a prime target for anti-cancer agents. However, the time that elapses between the loss of the telomerase activity and the induction of cell **senescence** can be lengthy if the tumor cell telomeres are long. This drawback has tempered the hopes raised by the direct targeting of telomerase, and has instead guided research toward the mechanisms involved in causing the shortening of the telomeres, with a view to targeting the telomeres of the cancer cells. Recent studies have shown that introducing the gene coding for telomerase (hTERT) into primary cells restores its expression and indefinitely lends the cells a **phenotype** of young normal cells (elongation of telomeres). The long-awaited elixir of life? Not so soon. But this result does raise an interesting question: might grafting **transgenic** cells overexpressing telomerase be useful in therapy, or would such a graft actually be dangerous and be the equivalent of a graft of tumor cells?

Laure Sabatier





INSERM

Normal fibroblasts (left) and transformed fibroblasts (right). The transformed cells cross over each other instead of lining up side by side.

lation (box E, ***The cell division cycle: duplication under control***). In senescent fibroblasts, the more the cells divide, the longer are phases G1 and G2 of the cell division cycle; the cells increase in size and their energy **metabolism** is disturbed; Senescent cells are characterized by a loss of proliferative capacity due first to a reduction of their ability to repair themselves (see *The Caretakers of the Genome*), and second to the gradual shortening of telomeric sequences. Various studies suggest that the loss of these sequences at each division may contribute to the appearance of the observed anomalies, the alteration of the **telomeres** destabilizing the ends of the chromosomes and leading to chromosome loss. In fact the set of chromosomes that forms the karyotype of senescent cells presents anomalies such as translocations and disappearance of chromosomes. This permanent loss of telomeric sequences also reduces the viability of these cells and ultimately causes their death.

A study of human embryo lung fibroblasts has shown that the senescence phase is characterized by the evolution of the cells toward **polyploidy** (multiplication of the number of chromosomes in the cell by two, three or *n*) associated with a high level of chromosomal anomalies. One hypothesis is that cell senescence may be accompanied by **clonal** chromosomal rearrangements causing cell transformation when certain **genes**, **oncogenes** or **tumor suppressor genes**, are present.

Instability «pre-cancerous» subjects

In patients with pre-cancerous illnesses a chromosomal instability culminating in telomeric fusions is often observed at pre-malignant stages or in tumors with low-grade malignancy. More advanced malignant tumors show clonal rearrangements, i.e., that are present in many tumoral cells, together with specific chromosomal imbalance.

In patients with illnesses such as *ataxia telangiectasia, xeroderma pigmentosum*, Bloom's syndrome or Werner's syndrome, the cells show a high level of spontaneous chromosomal instability (translocations, **inversions**, etc.), some involving specific chromosomes. The incidence of cancer among these patients is also very high, and they are hypersensitive to radiation. They present misrepairs and faulty recombination, the cell division cycle **control pathways** thus creating and maintaining the DNA damage responsible for the chromosomal instability. Both spontaneous and radiation-induced instability is high in these patients' cells.

In the case of *ataxia telangiectasia and xeroderma pigmentosum*, there seems to be no correlation between the size of the telomeres and the incidence of terminal dicentric chromosomes.

The cells of patients with Werner's disease, characterized by premature aging, display strong chromosomal instability. Faulty **replication**, **segregation**, repair or **transcription** can cause the accumulation of **mutations** and chromosomal anomalies. Patients with *progeria*, a genetic illness characterized by very fast aging, also show chromosomal instability with telomeric shortening.

Chromosomal instability in cancer cells

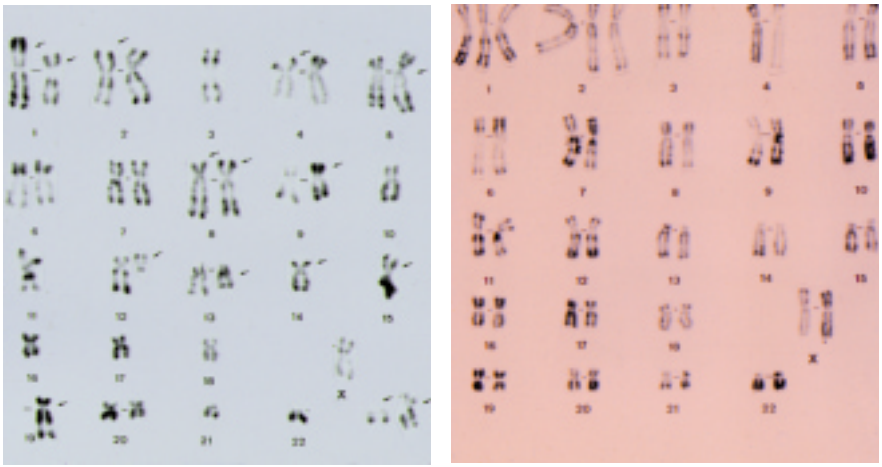
A tumor is characterized by an abundance of genetic alterations acquired in the course of a long clonal evolution (box 2). A normal cell acquires transformation features after one or more mutations have occurred that lend it a strong «proliferative edge».

Cancer implies a lifting of the normal constraints on cell proliferation. This is directly governed by the cycle mechanisms or indirectly through the control of the cell's engagement in terminal **differentiation** or in programmed cell death, called **apoptosis** (see *Cell Suicide*).

The normal regulating genes fall into two categories: those whose products stimulate cell proliferation, and those whose products inhibit it. The uncontrolled proliferation that characterizes cancer is steered in one of two ways: either a hyperactive proliferation stimulator gene (**dominant** mutation) is made, and the altered gene will be an oncogene (the normal **allele** being a **proto-oncogene**), or a proliferation inhi-

Cancer and aging 2

About 10^{16} cell divisions take place in the human body in the course of a lifetime. In an environment devoid of **mutagenic** factors mutations still occur spontaneously at a rate estimated at 10^{-6} mutations per gene per cell division. But a single mutation is not enough to transform a healthy cell into a cancerous one. The evidence shows that cancer formation requires the co-occurrence of several rare and independent events. Aging thus plays an important role in the accumulation of mutations, causing an increased incidence of cancer with advancing age. Statistical data show that between three and seven independent events are needed to transform a normal cell into a cancerous one. Leukemia needs the fewest events, carcinomas the most.



INSTIITUT CURIE

The four main cytogenetic cancer types. Left to right and top to bottom: monosomic (fibrosarcoma), trisomic (tumor of the uterus), translocation (between chromosomes 9 and 22 in leukemia), and normal karyotype.

biting gene is inactivated (**recessive** mutation); this will be called a tumor suppressor gene.

Gene amplification is one manifestation of genomic instability often observed during tumor growth. It can originate in a gain of chromosomes due to rearrangements. Or it can result from an intra- or extra-chromosomal mechanism, two forms of amplification that do not coexist in a tumor cell.

Cytogenetic studies provide a basis for classifying cancers into four types. Each one is characterized by specific profiles of chromosome imbalance, comprising modifications in both number and structure:

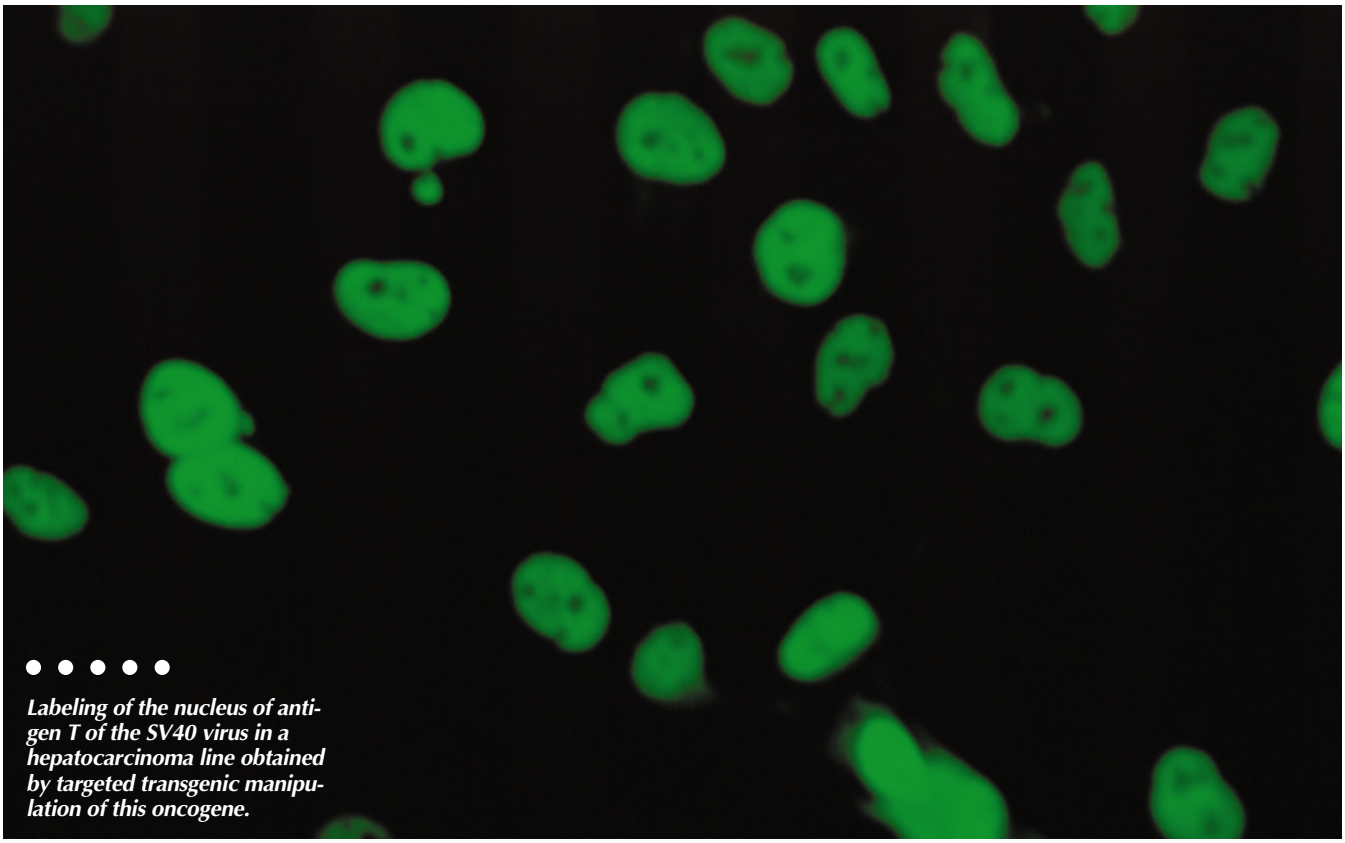
- The **monosomic** profile results from losses or **deletions** of chromosomes leading to transformation of the **diploid** cell into a **hypodiploid** cell. **Endoreduplications** can occur, giving rise to **hypotetraploid clones** with highly rearranged karyotypes. Thus the loss of chromosomes or chromosomal fragments reveals recessive mutations by deletion of normal alleles. This type of profile is found in particular in cancers of the breast, colon, lung and prostate.
- The **trisomic** profile features duplications of chromosomes with few rearrangements. These tumors evolve toward **triploidy**. This profile is that of **adenomas** and certain **carcinomas** such as **neuroblastomas** or Wilm's tumor.
- The **translocation** profile is characterized by reciprocal translocations without loss of genetic material. It is found in **hemopathies** and in some **sarcomas**.
- A few tumors have a **normal karyotype**. Their clinical and biological characteristics are close to those of tumors of the trisomic type.

Instability and cell immortalization

Normal human cells grown in culture have a limited lifespan. But if these cells are **transfected** with certain agents, e.g., **antigen** T, their cycle is modified and they become immortal. They exhibit a strong chromosomal instability that generates chromosomal imbalance, which when associated with an increased incidence of **aneuploidy** causes the line to become immortal.

To study the instability linked to this process healthy normal cells are transfected with an immortalizing agent such as SV40, a DNA virus that is non-pathogenic in humans. Antigen T is an oncogene; it codes for a phosphoprotein necessary for the replication of viral DNA. In healthy cells it operates by direct interaction with protein p53, coded for by the tumor suppressor gene, the one most frequently found to be mutant in human tumors. Antigen T prevents protein p53 from binding with DNA. Proteins p53 and pRb then no longer play their normal role of «brakes» (negative regulation of the cell cycle). The repair **enzymes** are disturbed, and lesions go unrepaired, generating increased numbers of chromosomal anomalies.

The cells transfected with antigen T of SV40 live longer than normal senescent cells. They go on dividing for longer, skipping a first mortality phase called M1 in which normal cells start to senesce and die. Cells containing antigen T of SV40 overtake phase M1 owing to the inhibition of p53, which induces



Labeling of the nucleus of antigen T of the SV40 virus in a hepatocarcinoma line obtained by targeted transgenic manipulation of this oncogene.

INSERM/U445

the onset of normal cells in senescence. These transfected cells carry on dividing up to a mortality phase M2, termed the «crisis» phase, when a number of them die. Those that survive are immortalized and go on dividing indefinitely. Cytogenetic data show that the instability of the fibroblasts transformed by antigen T is very high before and during the crisis. It is characterized by a large number of dicentric chromosomes, the level of which peaks during the crisis, when it accounts for the majority of the anomalies. After the crisis the level declines but stays predominant. The emerging clones exhibit chromosomal imbalance that lends the cells a proliferative advantage. This model makes it possible to follow the time course of the appearance of chromosomal imbalance generated by instability and the selec tion of the rearrangements made in the newly immortalized cells.

Radio-induced instability

The irradiation of cells induces a destabilization of the **genome** accompanied by a high cell mortality in the first mitotic divisions after the exposure to radiation (Figure). Some cells manage to survive for a few further generations. However, the incidence of dicentric chromosomes observed per cell halves at every generation.

It is therefore difficult to imagine finding chromosomal rearrangements several generations after irradiation. Even so, a radio-induced chromosomal instability has been observed in human **hematopoietic** cells (also in mice), in primary fibroblasts and in **lymphocytes**.

The cells that survive the irradiation produce offspring that possess a marked ability to form new chromosomal aberrations. These delayed effects of radiation can be considered as manifestations of a transmissible genomic instability. Thus stable damage induced by irradiation is slow to appear.

The period of instability is followed by the selection of clones characterized by a chromosomal imbalance that gives them a proliferative advantage, the consequence of which is the invasion of the culture by these clones. These anomalies are characteristic of the transformed or tumoral cells, and their lifespan is extended.

«Delayed» cell death has been observed with a lag of up to 30 or 40 generations in Chinese hamster ovocytes after treatment with X-rays, ethylmethanesulfonate or a **restriction enzyme**, but not after ultraviolet light treatment. Ultraviolet radiation, which is not ionizing, causes only single-strand damage, which suggests that the instability linked to postponed cell death may be due to the misrepair of double-strand breaks induced by radiation. The incidence of mutations at specific ***loci*** is increased in the offspring of irradiated cells. Anomalies detected in the transformed populations of mouse embryo cell lines are not a direct consequence of the radiation; the incidence of transformation is the same regardless of how many cells are irradiated.

All these findings demonstrate the existence of radio-induced chromosomal instability like that observed during cell aging. Irradiation thus prolongs the lifespan of cells and delays the process of cell senescence.

An essential step after stress

Chromosomal instability is an effect observed after various sorts of stress such as irradiation, immortalization, aging, or cancer formation. This insta-

bility seems to be an essential step at this stage for the cells to recover the stability they need to keep on growing.

When instability is highest some chromosomes seem to form dicentric aberrations more readily than others. A possible explanation that needs to be tested is that the chromosomes bearing the shortest telomeres are preferentially implicated in the formation of dicentric pairs. If the telomeres are short, the end of the chromosome is not stable and the chromosome can become stable only by fusing with another one. Chromosomal instability may thus be a necessary mechanism for a diploid cell to become a transformed immortalized aneuploid cell, irrespective of the type of stress involved.

Françoise Hoffschir
and **Laure Sabatier**
Department of Radiobiology and Radiopathology
Life Sciences Division
CEA/Fontenay-aux-Roses - France

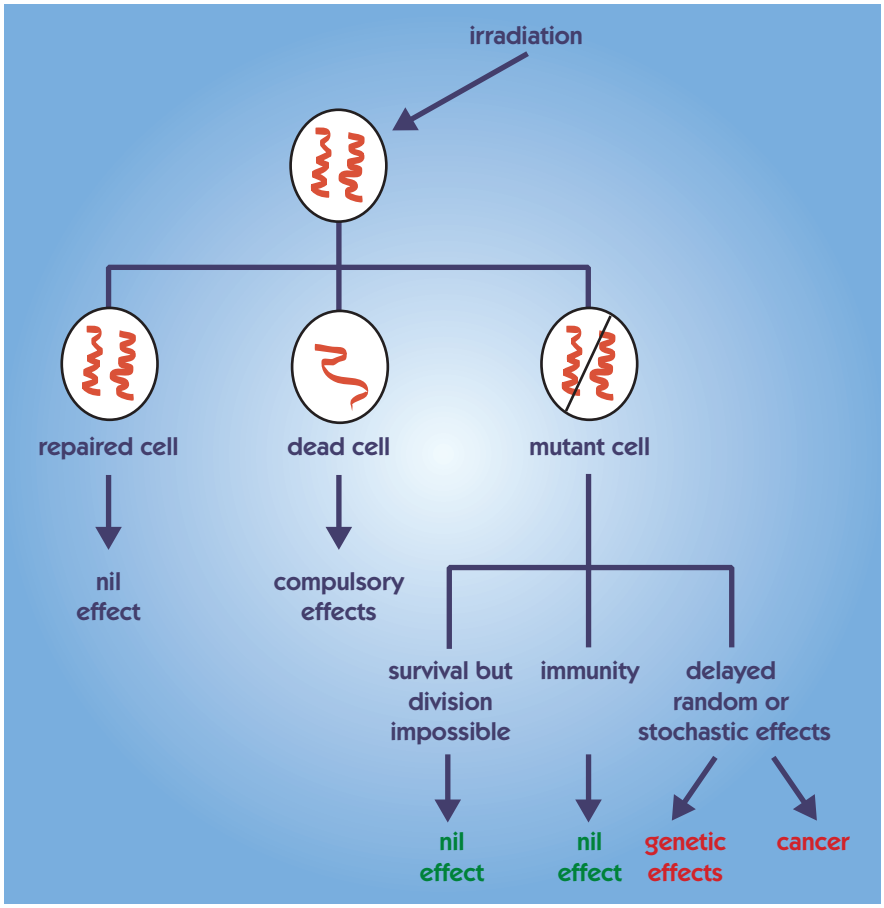


Figure. Impact of irradiation on cells. In most cases irradiation has no lasting effect on the cell, unless the cell mutates, gives rise to mutant daughter cells, and then engages in a tumor growth process.