université PARIS-SACLAY | FACULTÉ DE PHARMACIE

Chemical carcinogenesis

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Learning objectives

1. Define genotoxic and non-genotoxic carcinogens
2. Know the three stages of carcinogenesis: initiation, promotion, progression
3. Describe the phenotype of a tumour cell
4. Understand the role of oncogenes, the hallmarks of cancer
5. Explain the mechanisms of non-genotoxic carcinogenesis and give examples of molecules.

Important 

Agenda

1. **Definitions**
2. **Stages of carcinogenesis**
   - Initiation
   - Promotion
   - Progression
3. **Tumoral cell phenotype**
4. **Oncogens**
5. **Non genotoxic (epigenetic) carcinogens**
6. **How to evaluate carcinogenic effects?**

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS CLASSIFICATION

The classification indicates the level of certainty that a substance can cause cancer (*hazard identification*)

IARC Group	Level of certainty that a substance can cause cancer (typical examples of evidence leading to each group)	Substances evaluated
GROUP 1	**CARCINOGENIC TO HUMANS** Sufficient evidence in humans. Causal relationship established.	Tobacco smoking, solar radiation, consumption of alcoholic beverages, consumption of processed meat, benzene, ionizing radiation, outdoor air pollution, asbestos
GROUP 2A	**PROBABLY CARCINOGENIC TO HUMANS** Limited evidence in humans. Sufficient evidence in experimental animals.	Emissions from high-temperature frying, glyphosate, DDT, consumption of red meat
GROUP 2B	**POSSIBLY CARCINOGENIC TO HUMANS** Limited evidence in humans. Less than sufficient evidence in experimental animals.	Gasoline engine exhaust, radiofrequency electromagnetic fields, *Aloe vera*, lead
GROUP 3	**NOT CLASSIFIABLE AS TO ITS CARCINOGENICITY TO HUMANS** Inadequate evidence in humans. Inadequate evidence in experimental animals.	Coffee drinking, crude oil, mercury, paracetamol
GROUP 4	**PROBABLY NOT CARCINOGENIC TO HUMANS** Evidence suggesting lack of carcinogenicity in humans and in experimental animals.	Caprolactam

Higher level of certainty

Lower level of certainty

This classification does not indicate the level of risk associated with exposure (*risk assessment*)

Only one substance in Group 4, because the IARC Monographs focus on substances that are suspected to cause cancer, based on scientific publications

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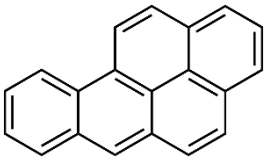
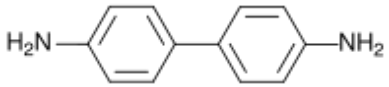




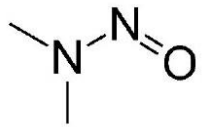


Figure 1: *N*- nitrosodimethylamine (NDMA)

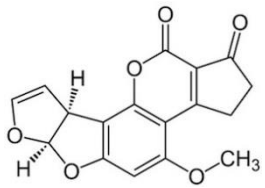


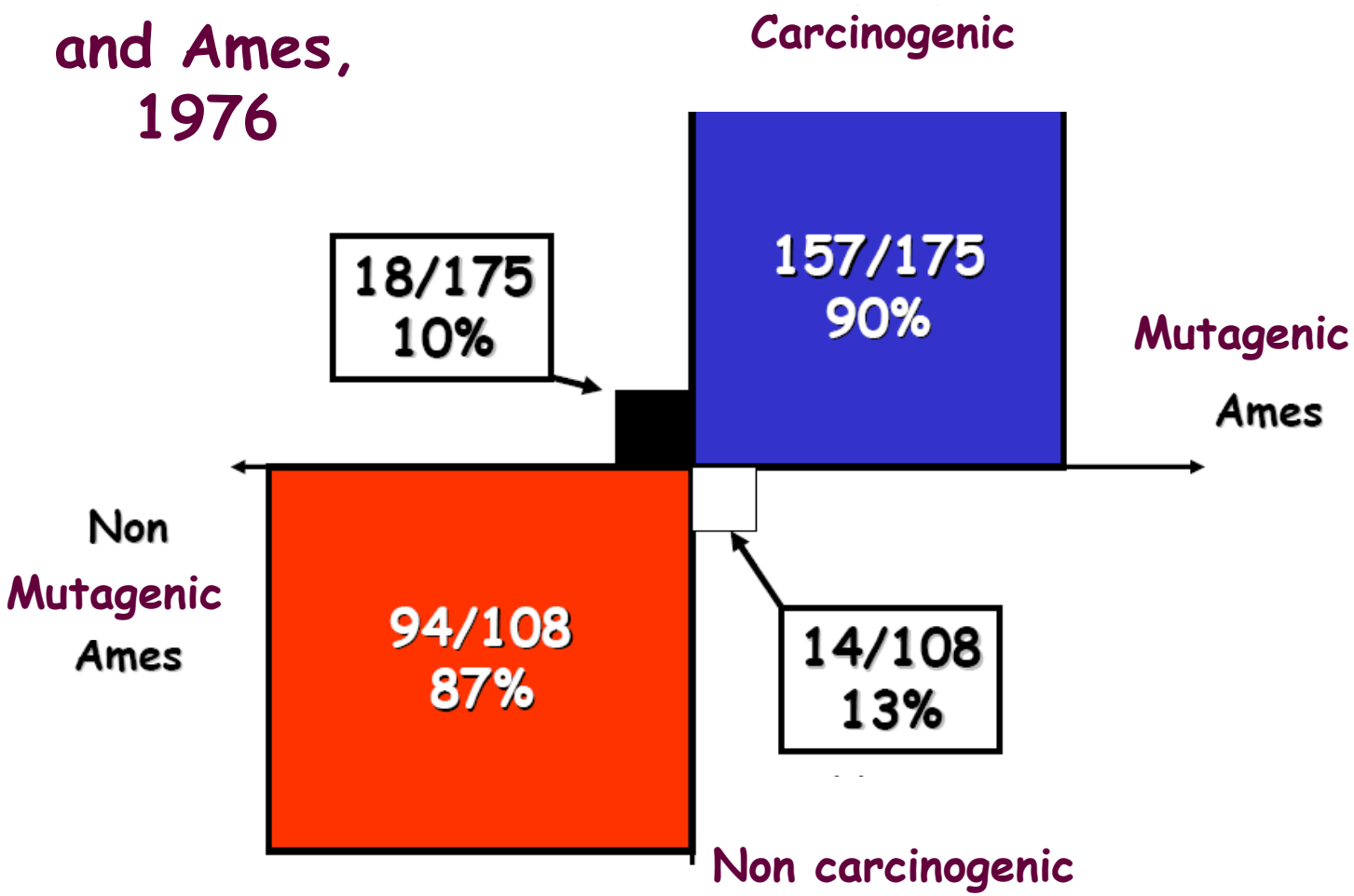
TABLE II
Chemical carcinogens.

Group	Compound	Mechanism of action	Affected organs/ Cancer type
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene Polychlorinated biphenyls (Luch 2005)	Form adducts with purine bases of DNA, mainly resulting on transversions	Skin, lungs, stomach Liver skin
Aromatic amines/amides	2-Acetylaminofluorene 4-Aminobiphenyl 2-Naphthylamine (Luch 2005)	Genotoxic compounds, increase the rate of cell duplication	Liver, bladder Bladder Bladder
Aminoazo dyes	o-Aminoazotoluene *N*, *N*-dimethyl-4-aminoazobenzene (Golka et al. 2004)	Forms adducts with DNA and with haemoglobin	Liver, lungs, bladder Lungs, liver
N-nitroso compounds	N-Nitrosodimethylamine (Drablos et al. 1998)	Form adducts at N- and O-atoms in DNA bases	Liver, lungs, kidneys
Carbamates	N-methylcarbamate esters (Wang et al. 1998)	Chromosome aberration, gene mutation, cell transformation	Experimental results showed liver, kidneys and tests degeneration
Halogenated compounds	Trichloroethylene (Lock et al. 2007)	Somatic mutations, modification of cell cycle pathways	Experimental results showed kidney, liver and lung cancer
Natural carcinogens	Aflatoxin B1 (Wild et al. 1986) Asbestos (Luch 2005)	Forms adducts with guanine, react with RNA and proteins	Liver Lungs
Metals	Arsenic (Shi et al. 2004) Cadmium (Hartwig et al. 2002) Nickel (Costa et al. 2003)	Oxidative stress Inhibit DNA repair pathways and nucleotide-excision repair Histone acetylation and DNA hypermethylation	Skin, lungs, liver Lungs, prostate, kidneys Lungs, nasal cavity
Anticancer drugs	Alkylating agents (Luch 2005)	Interstrand and/or intrastrand cross-links	Leukaemia

Oliveira *et al*, Annals of the Brazilian Academy of Sciences, 2005

Many mutagens are also carcinogens, but some carcinogens are not mutagens.

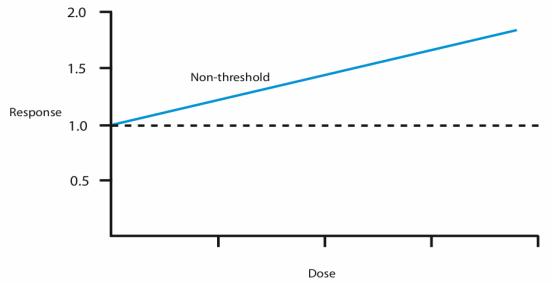
From Mc Cann and Ames, 1976



1 Definitions

- **Genetic carcinogens (genotoxic)**

- Detectable by mutagenesis studies.
- Causes **DNA damage**.
- All or nothing effect, no threshold.



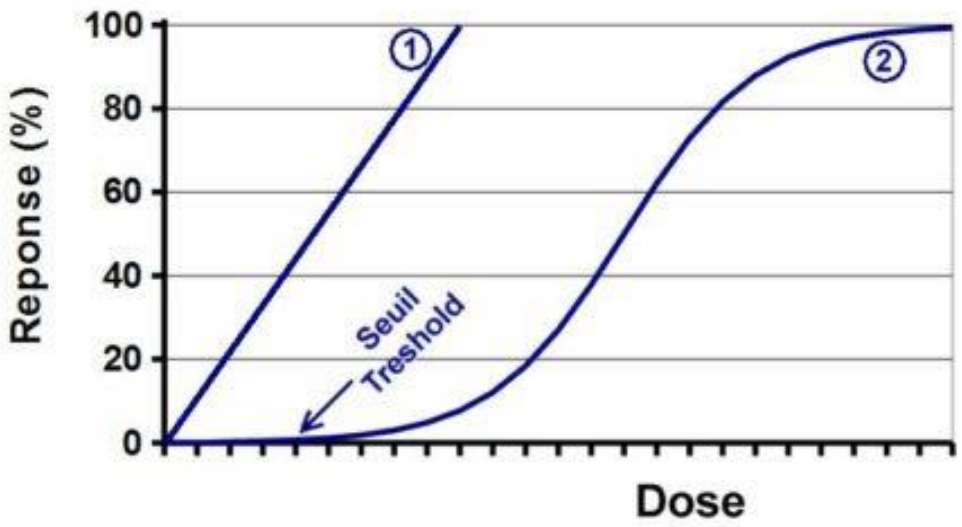
- **Epigenetic carcinogens**

- Not detectable by mutagenesis studies.
- Cause **no damage to the DNA**.
- Reversible modifications in the activity of genes, leading to a **modification of their expression**. Epigenetic processes are involved in the regulation of numerous events such as cell division, differentiation, survival, mobility, etc. The alteration of these mechanisms can favour the transformation of healthy cells into cancerous cells.
- **Dose effect, threshold, reversible**.

1 Definitions

Non-threshold toxic effects

These xenobiotics are essentially **genotoxic**.
For these substances, it is considered that the initiation of carcinogenesis or an effect on offspring is triggered by **mutations** in the genetic material (DNA).

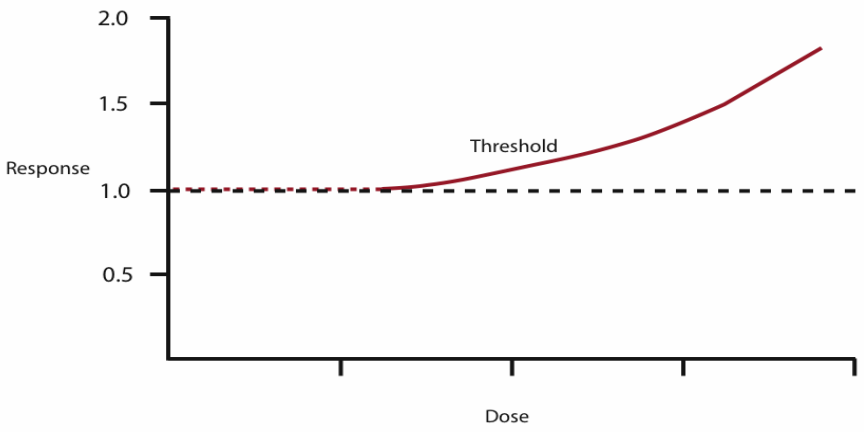


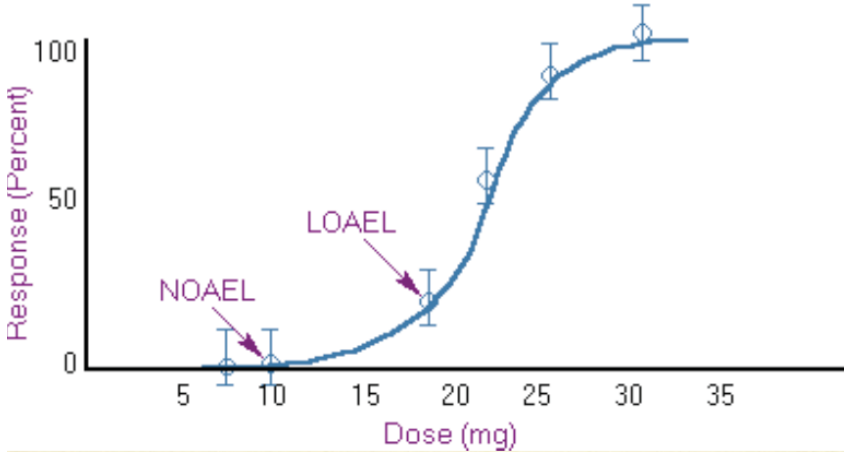
1 Definitions

Threshold toxic effects

They concern substances which, above a certain dose, cause damage whose severity is proportional to the absorbed dose.

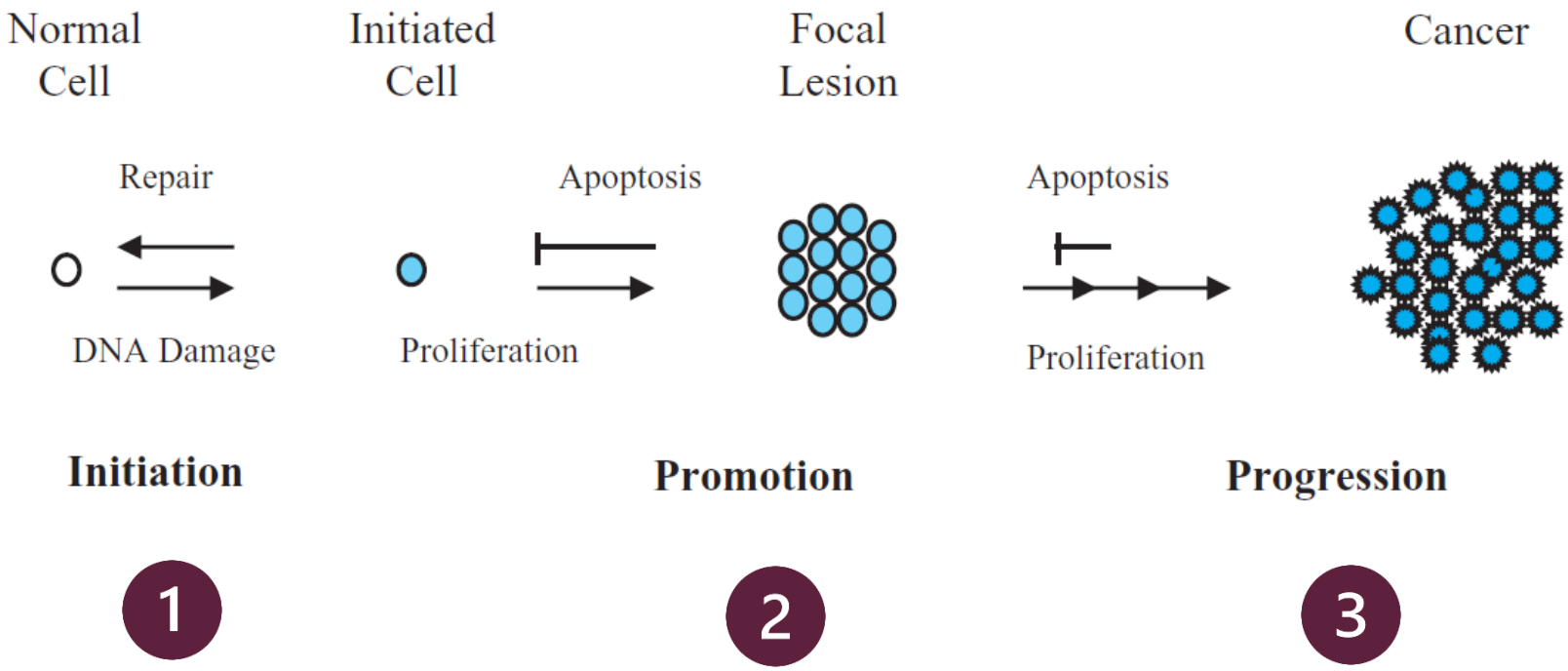
According to this classical approach, toxic effects only occur if this dose is reached and exceeds the detoxification, repair or compensation capacities of the organism.





NOAEL : no observed adverse effect level

2 Carcinogenesis stages



Casarett & Doull's

2 Carcinogenesis stages

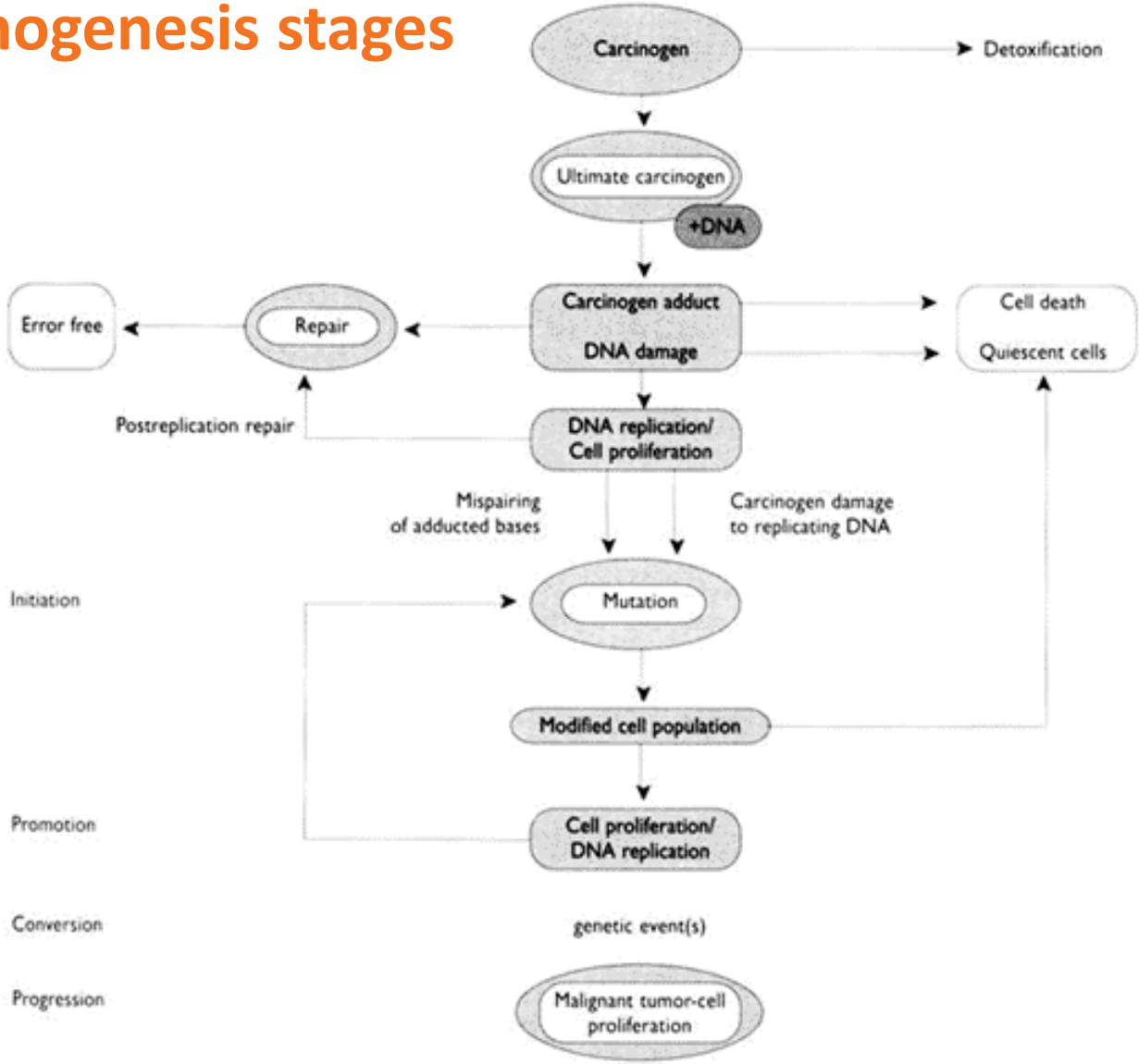


FIGURE 2. ROLES THAT CELL PROLIFERATION AND DNA REPAIR PLAY IN THE MULTISTAGE CARCINOGENIC PROCESS OF INITIATION, PROMOTION, CONVERSION, AND PROGRESSION (ADAPTED FROM IARC, 1992)[14]

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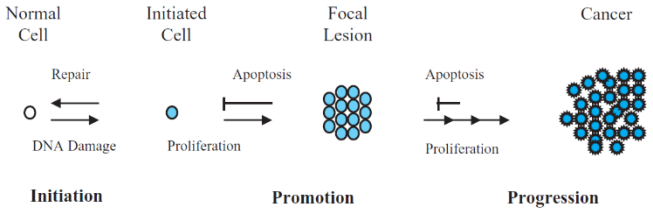
2 Carcinogenesis stages

TABLE 5. *Stages of carcinogenesis induced by specific agents*

Class[a]	Example	Stage (or stages)
I	Diethylnitrosamine, aflatoxin B_1, 2-naphthylamine, 2-acetylaminofluorene, methylcholanthrene, urethane, tobacco smoke	Initiation, promotion, progression
	Phenobarbital, tetradecanoylphorbol acetate, dietary fat and calories, ethanol	Promotion (progression)
	Prolactin, estrogens, and androgens	Promotion
	Foreign body, asbestos, benzene, potassium arsenite, diethylstilbestrol	Progression
II	Ionizing radiation (UVB and UVC)	Initiation, progression
	UVA radiation	Promotion
III	Papova, retro, and Epstein-Barr viruses	(Promotion) progression
	Herpes and hepadna viruses	Progression
IV	Transgenesis	(Promotion) progression
	Selective breeding	Initiation, promotion, progression

[a] From Table 1.



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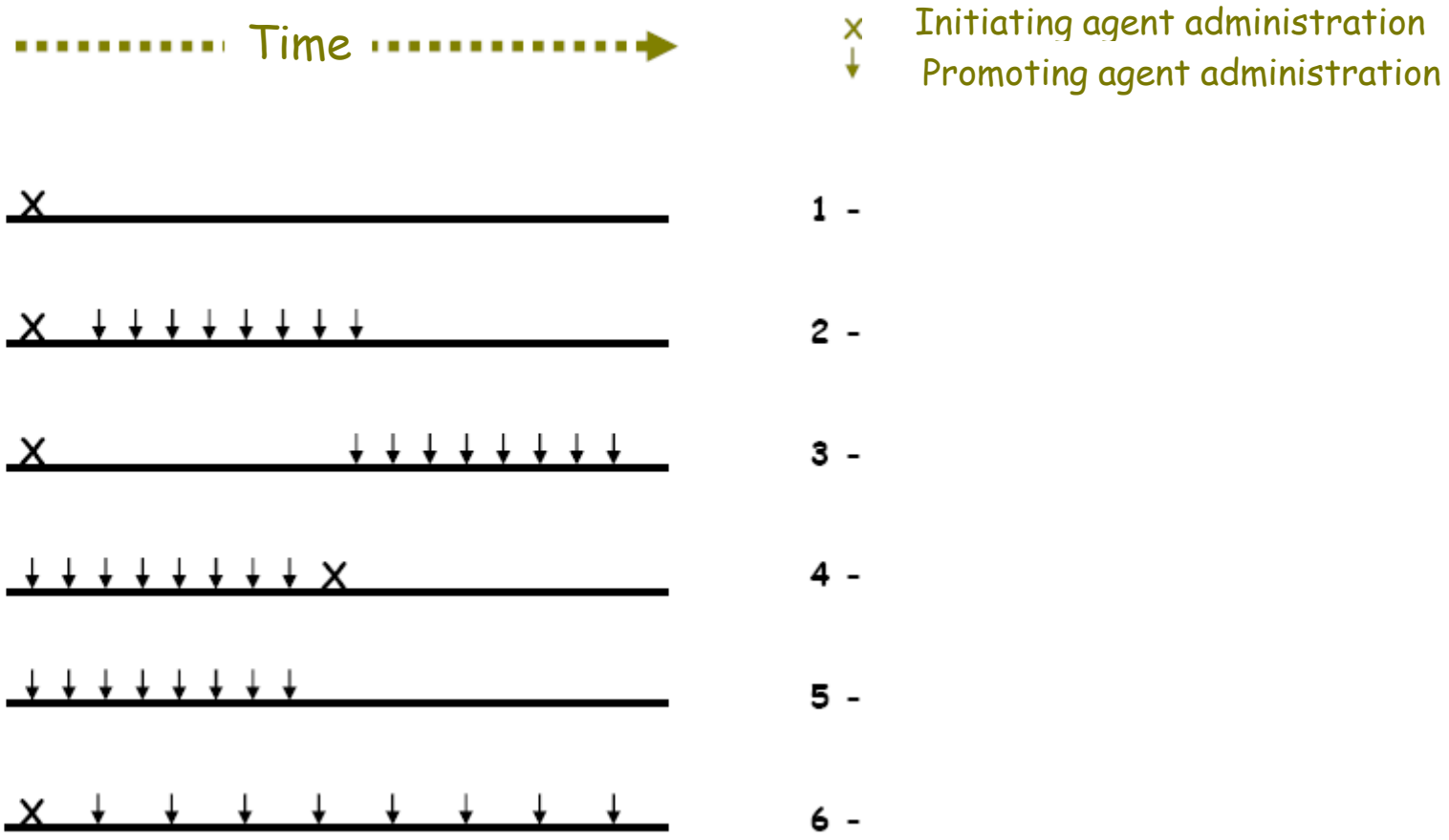
2 Carcinogenesis stages

Stage	Caracteristics
Initiation	Results from an **irreversible** genetic alteration, most likely one or more simple mutations, transversions, transitions, and/or small deletions in DNA.
Promotion	Does not involve changes in the structure of DNA but rather in the expression of the genes. **Reversible**.
Progression	**Irreversible** karyotypic instability and malignant growth

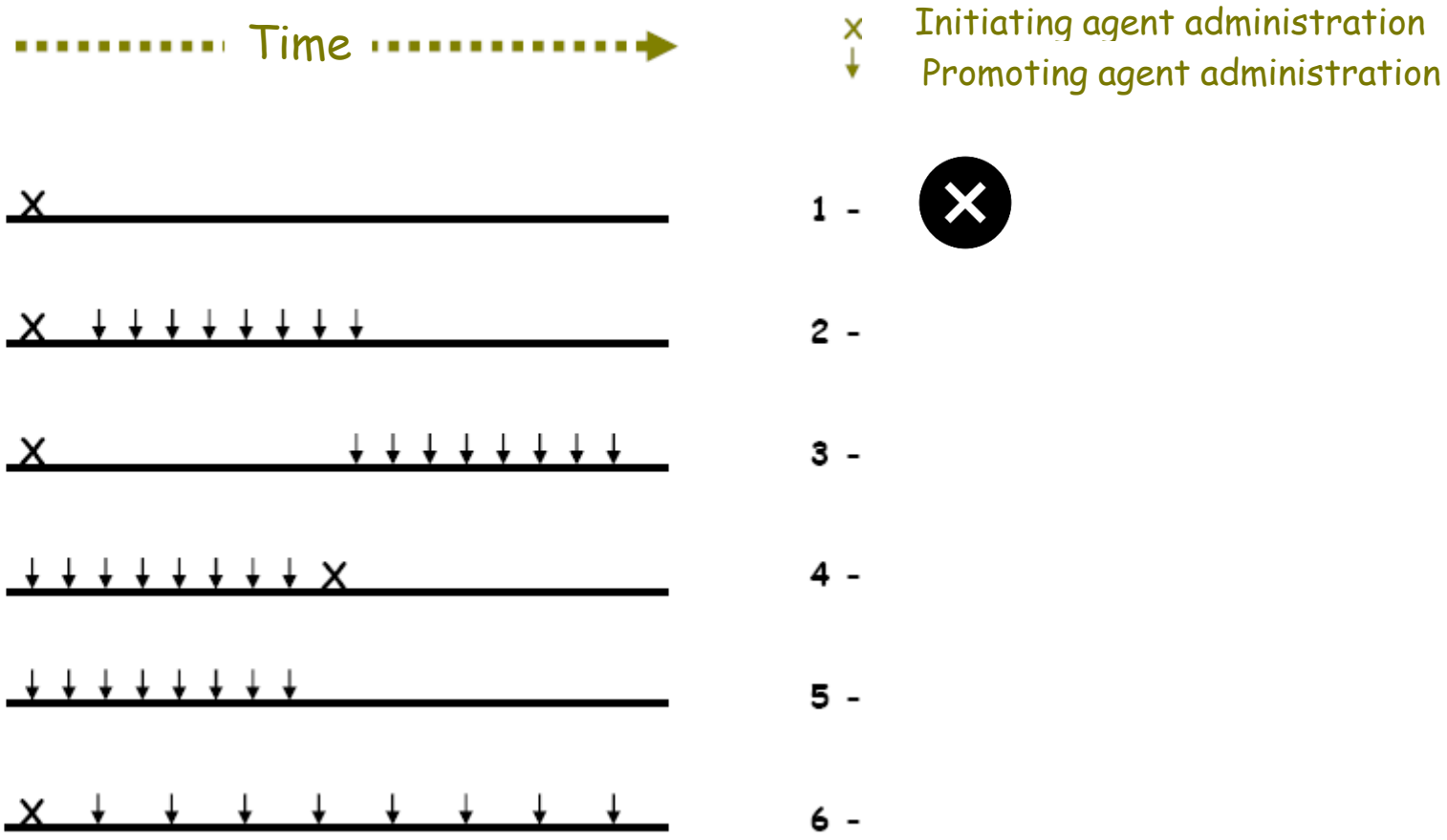
2 Carcinogenesis stages

Initiation promotion model



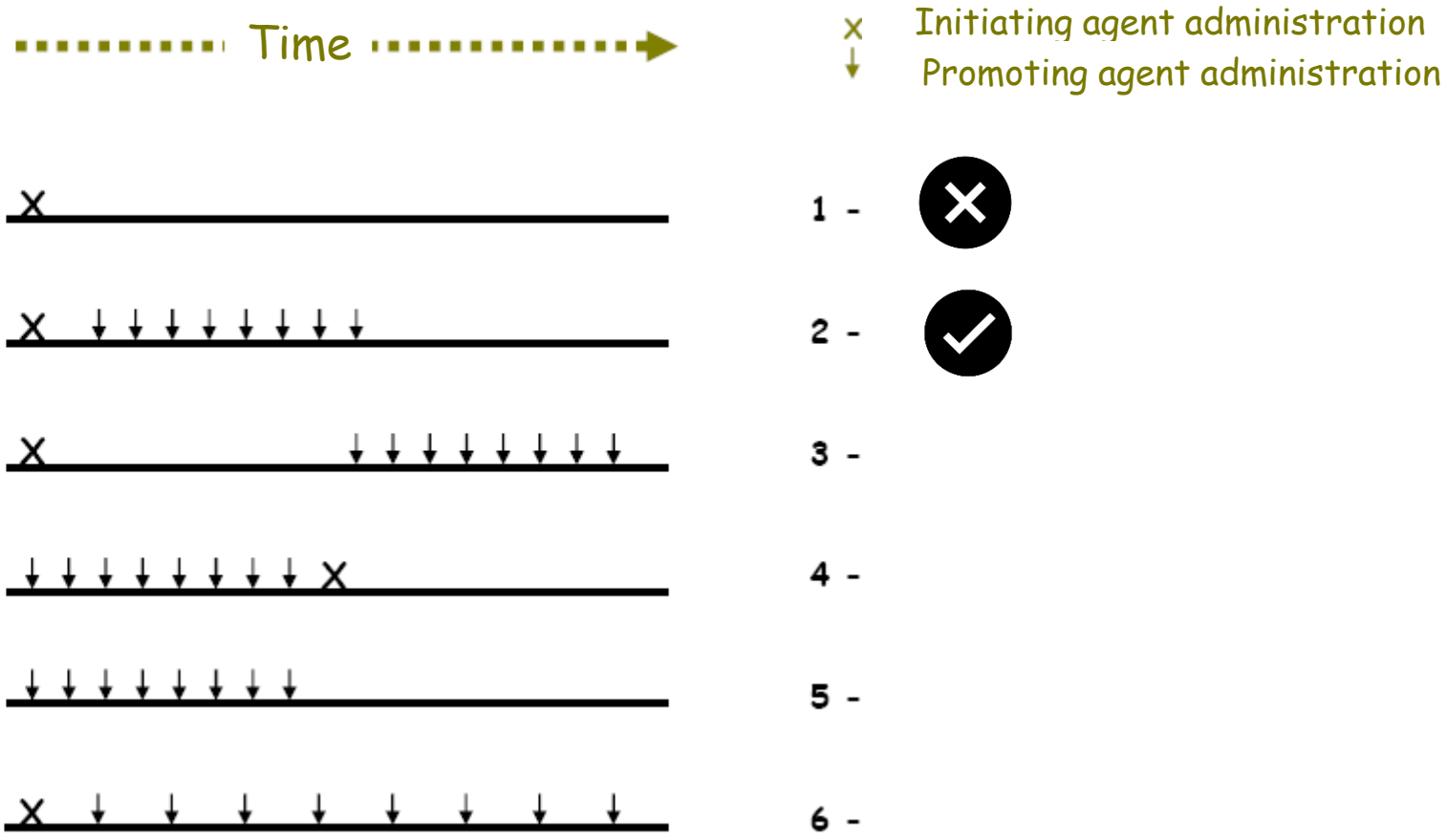
2 Carcinogenesis stages

Initiation promotion model



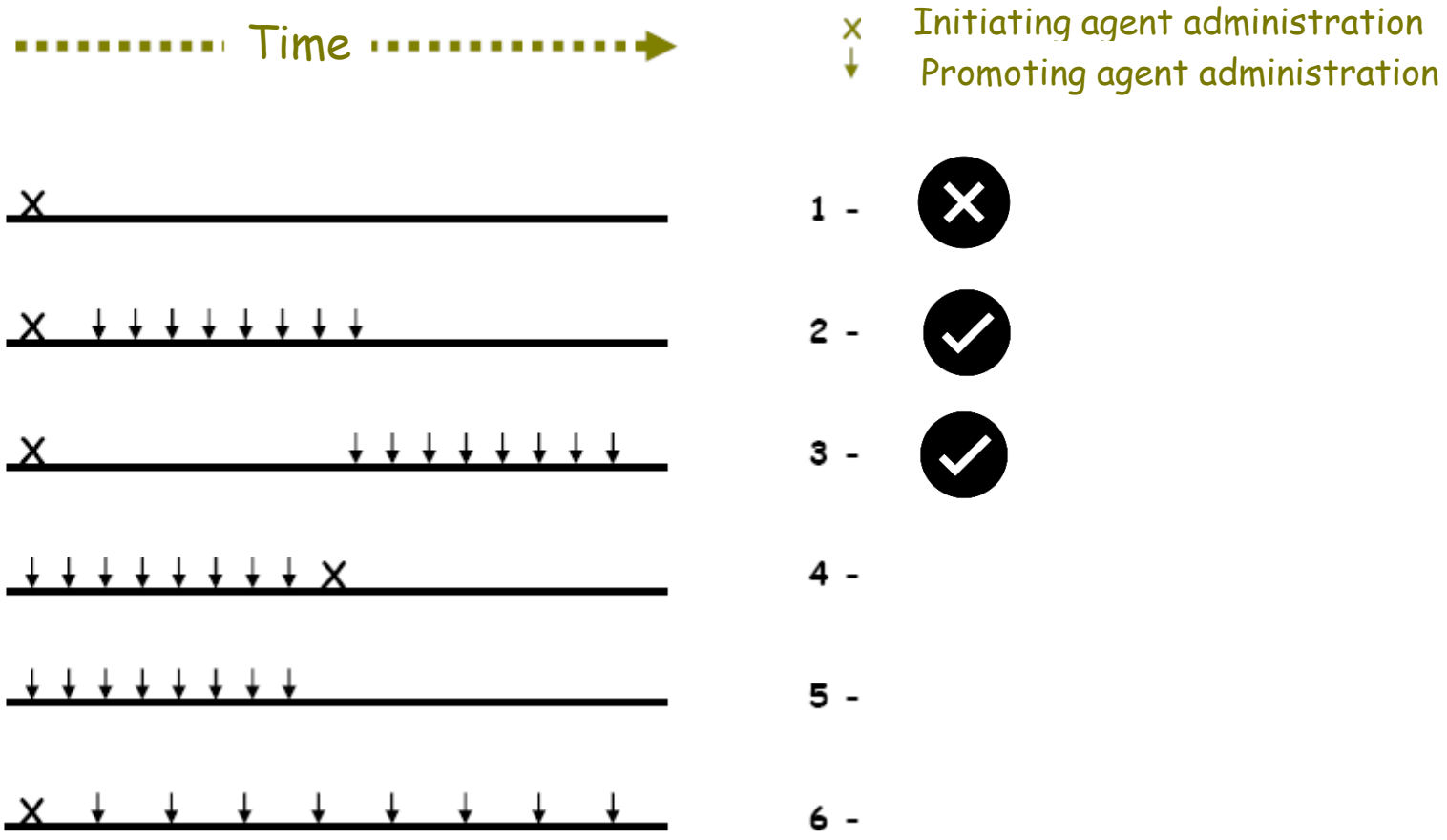
2 Carcinogenesis stages

Initiation promotion model



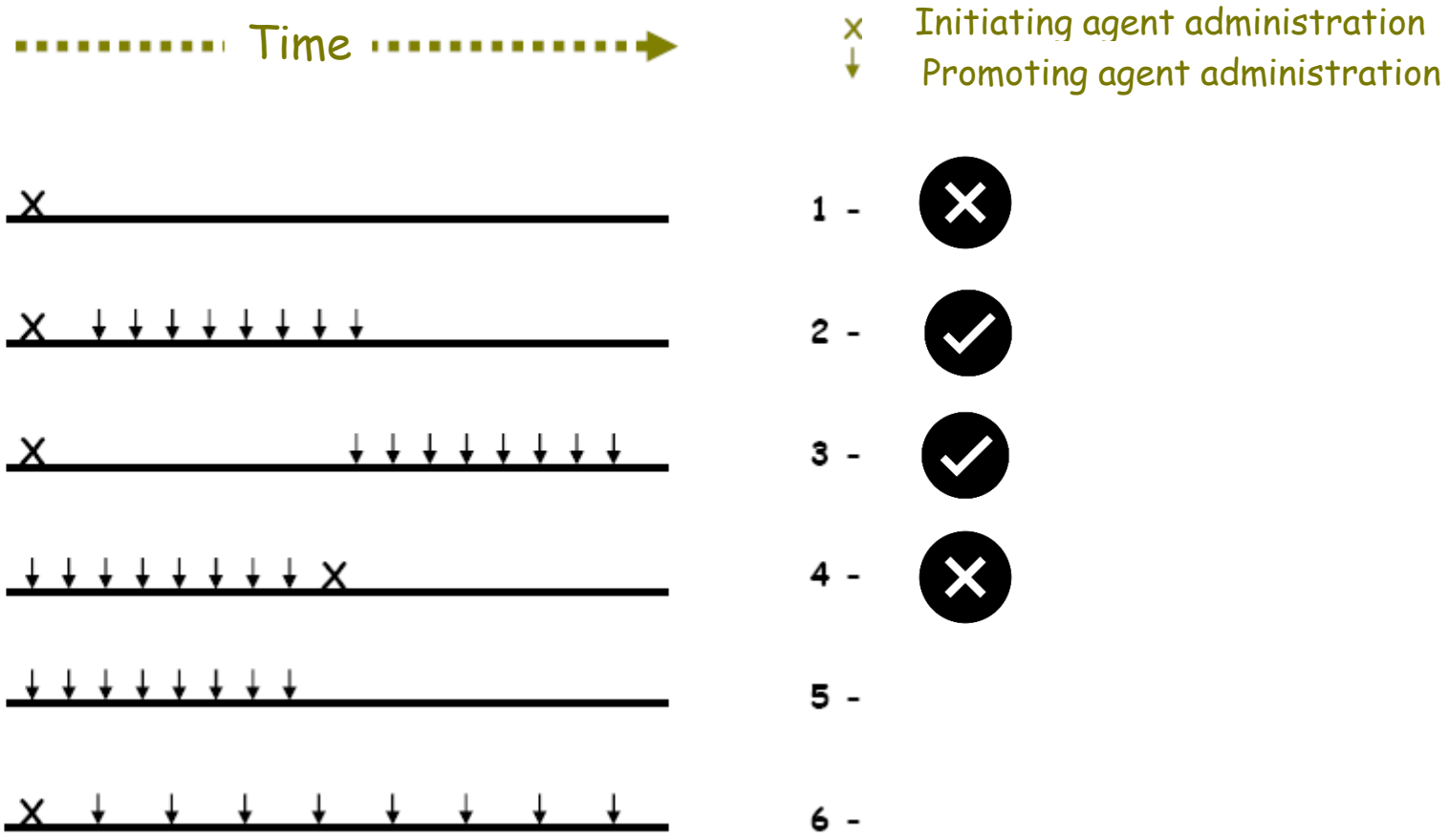
2 Carcinogenesis stages

Initiation promotion model



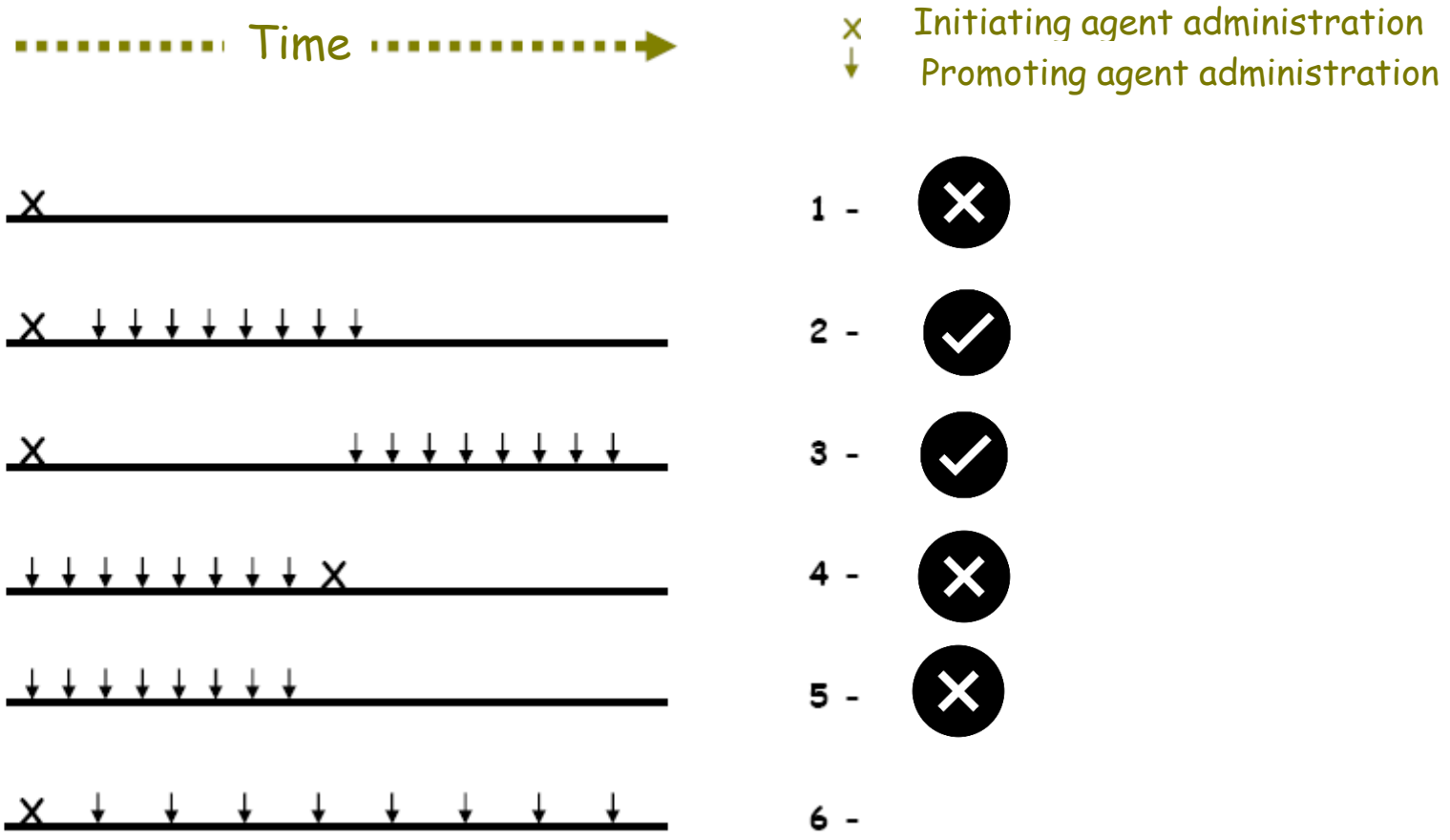
2 Carcinogenesis stages

Initiation promotion model



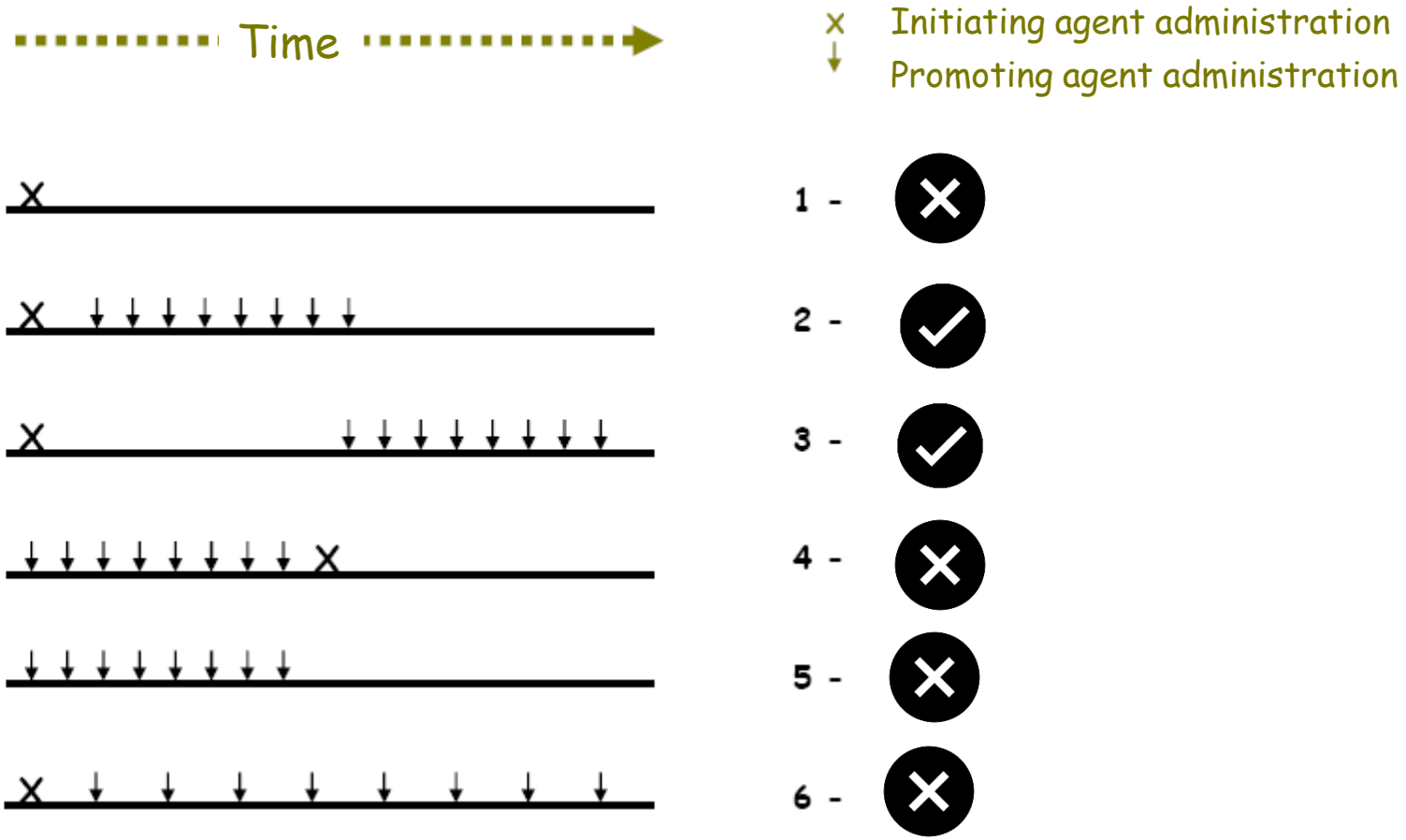
2 Carcinogenesis stages

Initiation promotion model



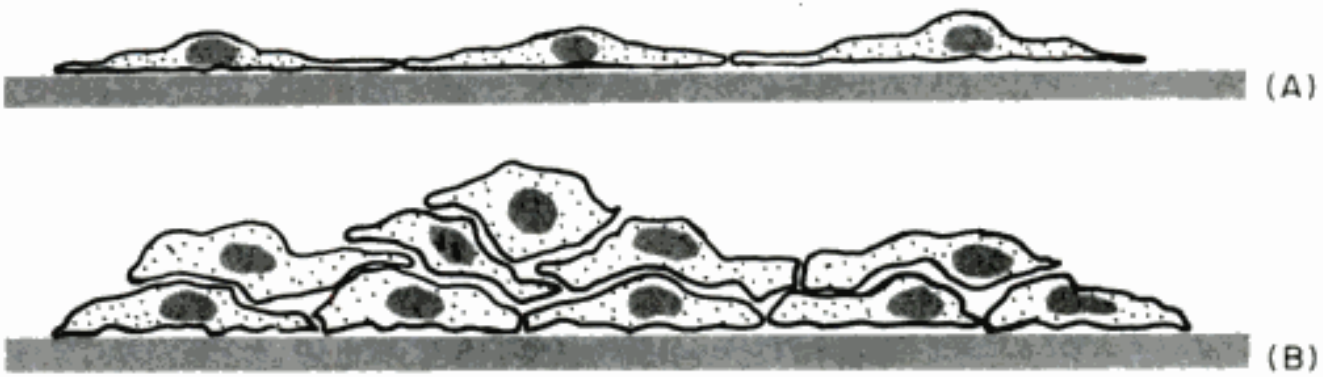
2 Carcinogenesis stages

Initiation promotion model



1. Loss of contact inhibition

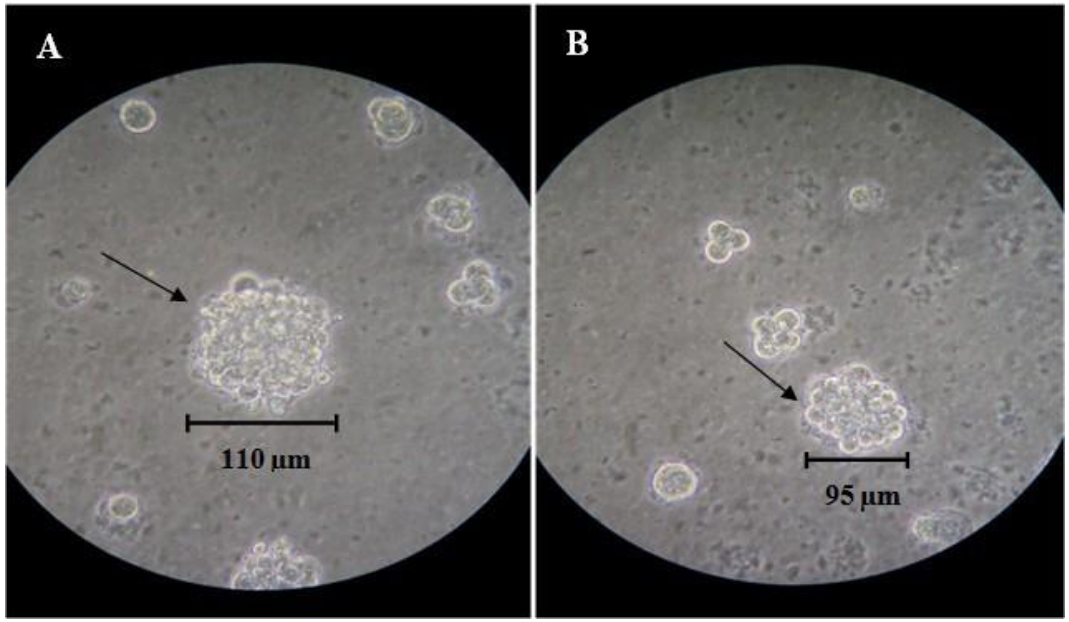
Development of proliferative foci



3 Tumoral cell phenotype

2. Ability to proliferate in the presence of low concentrations of growth factors.

3. Ability to grow in the absence of support
(development of colonies in semi-solid medium).

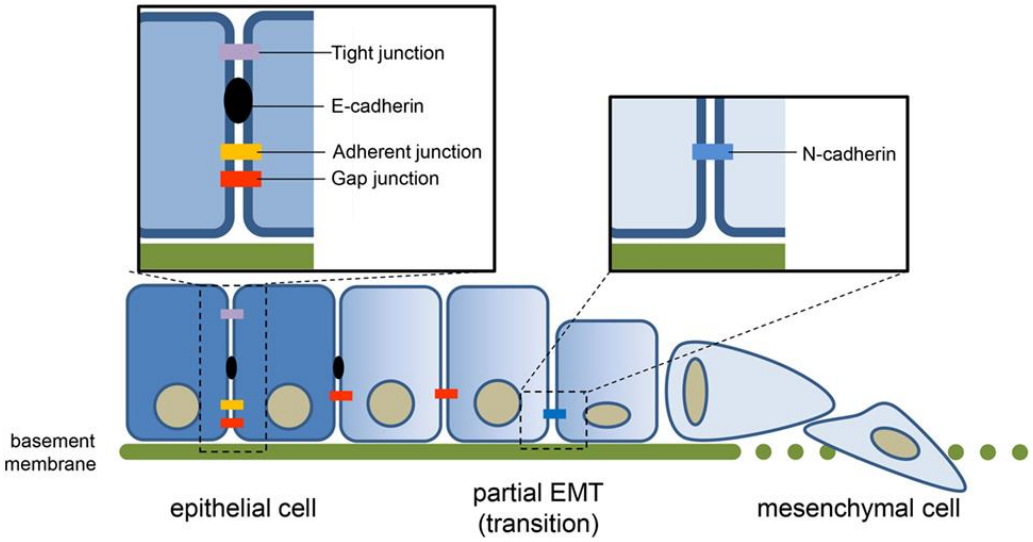


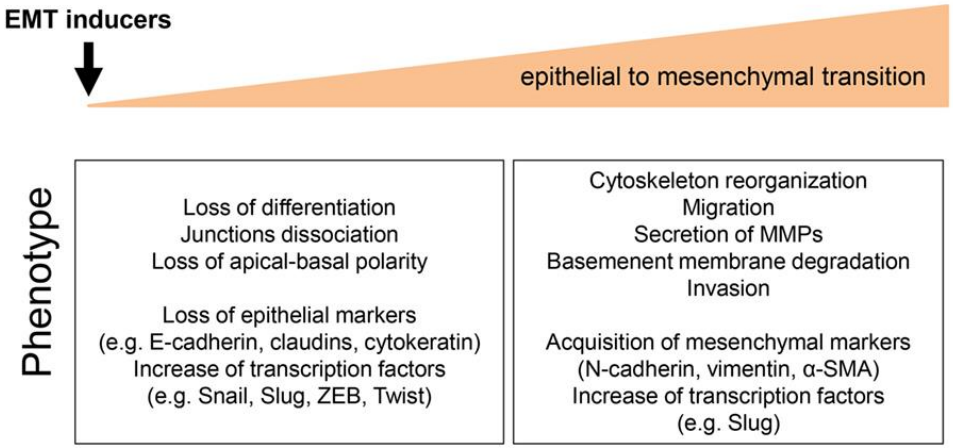
Soft-agar Colony Formation Assay (Clonogenic Assay) of the MBC1 (A) and MBC2 (B) Cell Lines at 14 Days after Seeding. Magnification, 10X.

Kamalidehghan *et al* 2012

4. Tumor formation in nude mice.

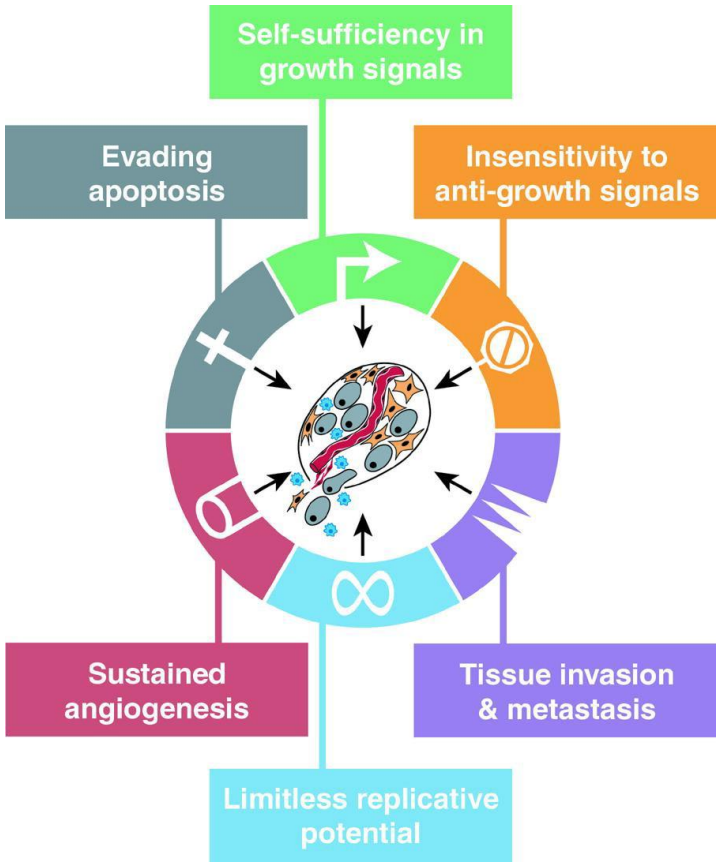
3 Tumoral cell phenotype
Epithelial-mesenchymal transition (EMT)





Morandi *et al*, Front. Oncol 2017

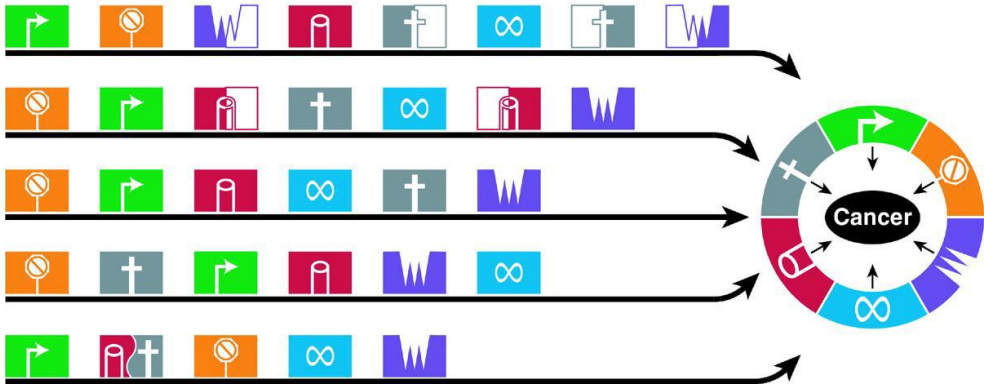
3 Tumoral cell phenotype



A

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

B



Hanahan and Weinberg, Cell, 2000.

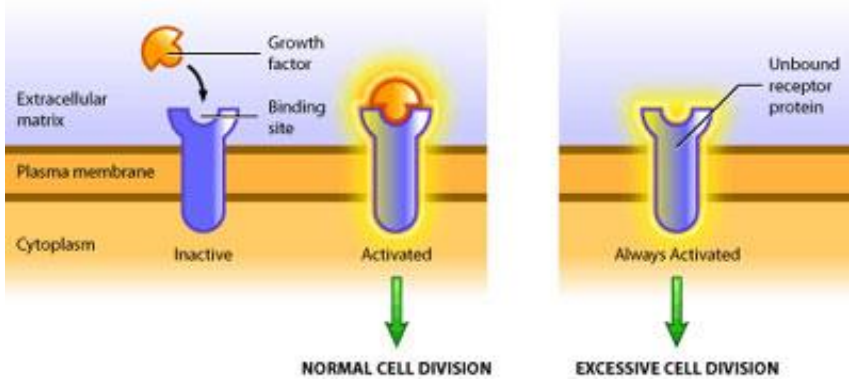
4 Oncogens

- Genes which, when expressed in a disordered manner or when their structure is altered, **contribute to the transformed phenotype of a cell**.

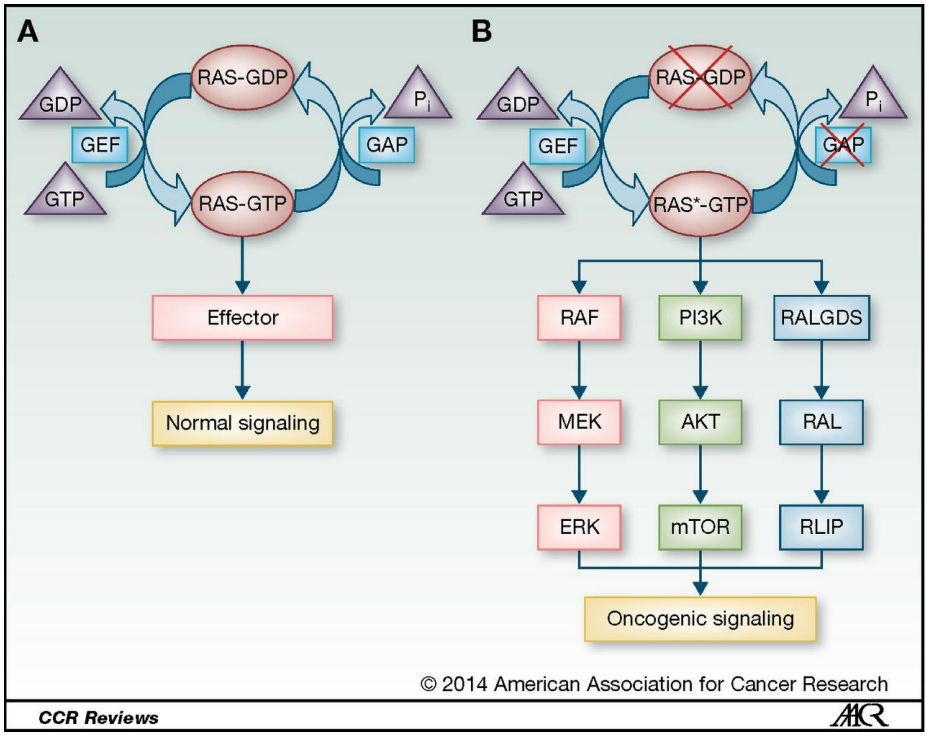
- Code for proteins with a wide range of functions (transcription factors, transcriptional activators or repressors, proteins involved in chromatin remodeling).

- Play a role in cell proliferation and survival such as the anti-apoptotic protein BCL-2, growth factor receptors, intracellular effectors of signal transmission such as proteins of the Ras family and intracellular tyrosine kinases.

4 Oncogens



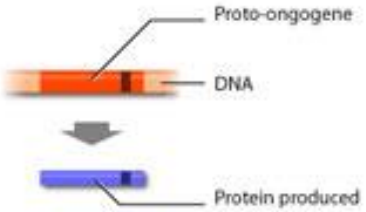
The Ras V12 mutation decreases the GTPasic activity of Ras, **keeping it in an active state related to GTP**.



Vasan *et al,* 2014

4 Oncogens

1. Deletion or Point Mutation in Coding Sequence: constitutively active protein produced in normal amounts

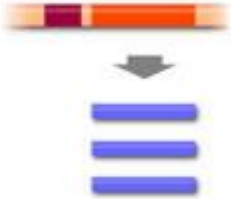


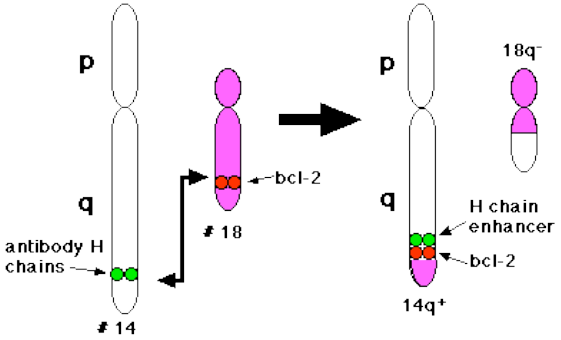
Ras V12

2. Gene Amplification: normal protein produced in much higher amounts

3. Chromosome Rearrangement:

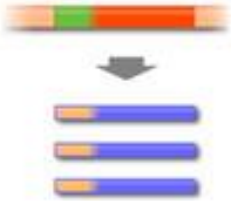
a) placement of strong enhancer nearby causes overproduction of normal protein

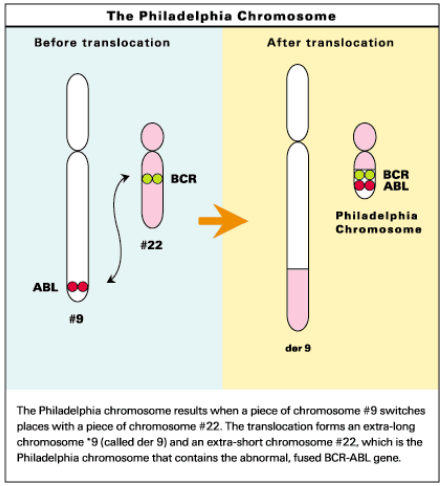




OR

b) fusion to another actively transcribed gene results in either increased levels of the fusion product (normal activity overproduced) or the fusion protein is hyperactive (increased activity in normal amounts)





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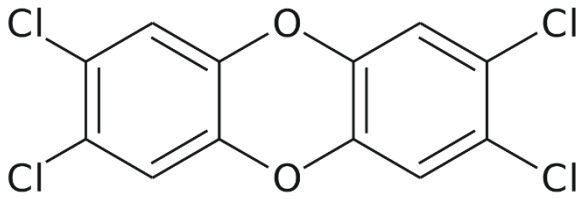
5 Non-genotoxic carcinogens

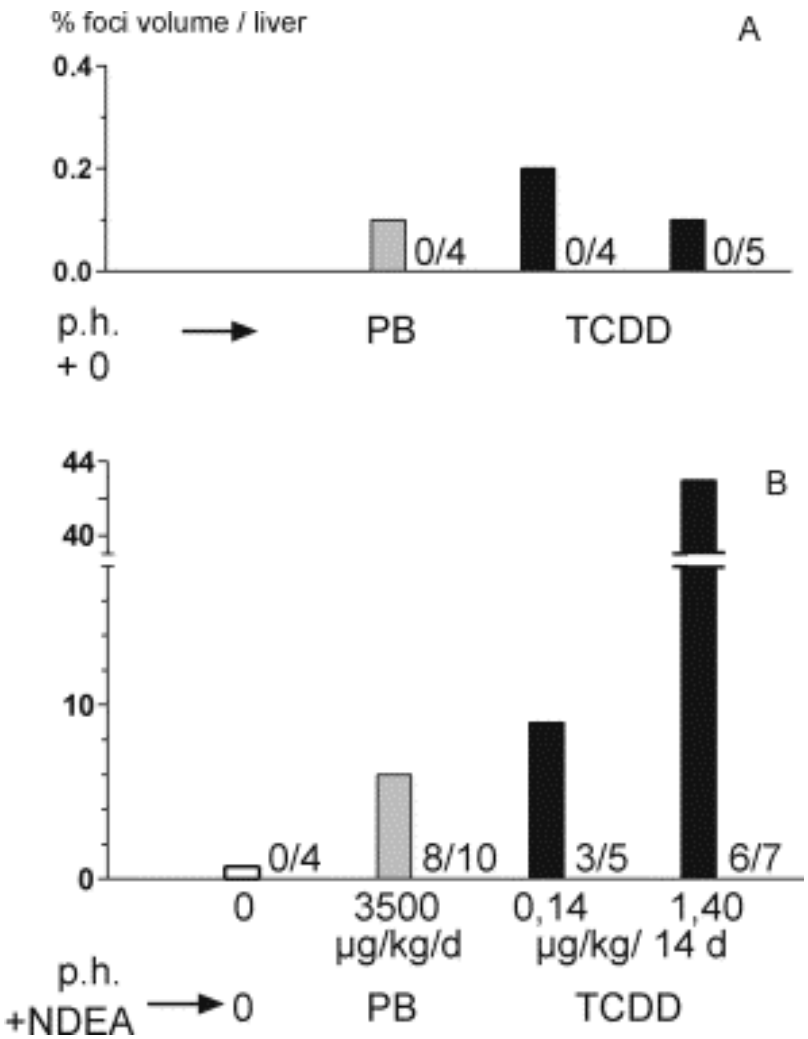
Many non-genotoxic agents are capable of inducing tumors in laboratory animals and are therefore considered as carcinogens.

Most of the chemicals belonging to this group were previously classified as **promoters**.



E.g. chlorinated polycyclic hydrocarbons such as **dioxin** (**TCDD**).

5 Non-genotoxic carcinogens



TCDD is a potent promoter of liver tumors in rats after initiation with N-nitrosodiethylamine (Pitot *et al*, 1980).

Liver tumor promotion by TCDD. Female Charles River rats were partially hepatectomized (p.h.) and treated 13 h later with (A) saline, or (B) with an initiating dose of 10 mg/kg NDEA by intragastric intubation.

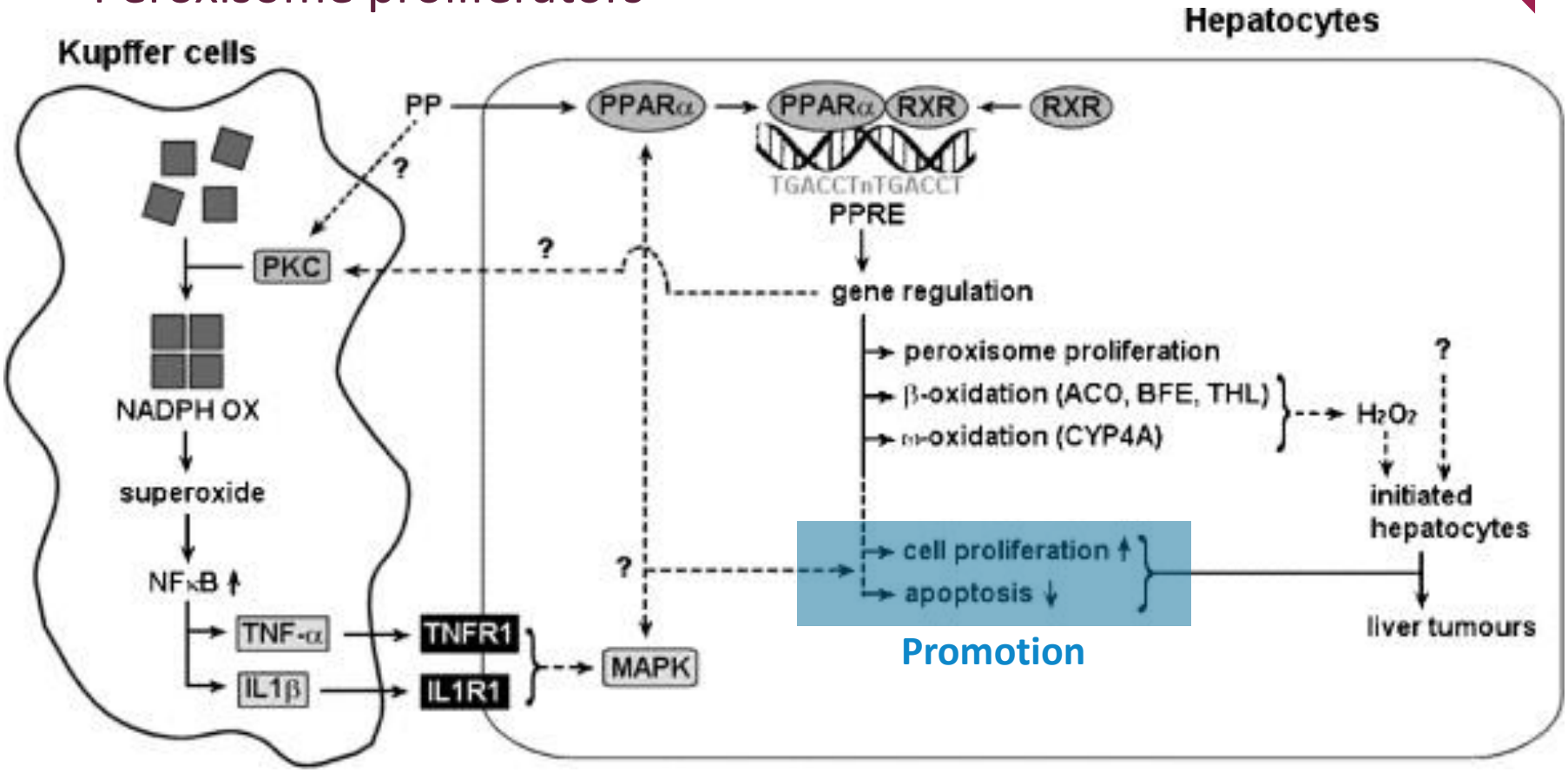
After **28 weeks** of treatment with **Phenobarbital**, Pb (0.05% in the diet) or **TCDD** (biweekly s.c. injections in corn oil) or **vehicle alone** (0), livers were examined by histomorphometry for development of altered hepatic foci.

Hepatocarcinoma and hepatic nodules were diagnosed by histopathological criteria and the sum of both is given right to the bars as number of rats with tumors/number of rats examined.

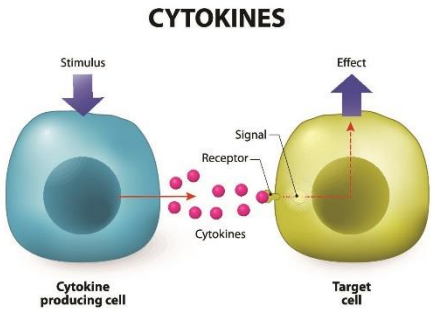
5 Non-genotoxic carcinogens
Peroxisome proliferators

Drugs	Pesticides	Chemical products
AspirineFibrates (fenofibrate, clofibrate…)	Diméthrine HaloxyFOP Lactofen	Dibutylphtalate Perchlorethylène Trichlorethylène

5 Non-genotoxic carcinogens
Peroxisome proliferators



Paracrine effect



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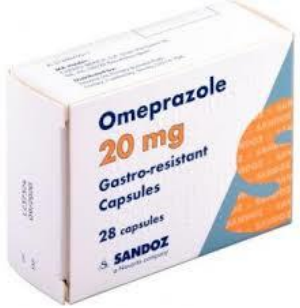
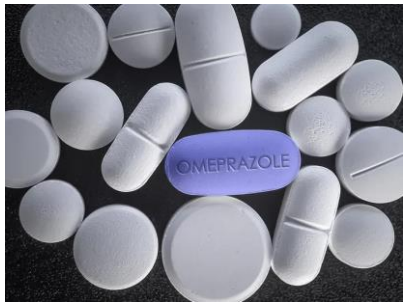
5 Non-genotoxic carcinogens
Omeprazole





- **Dosage**

20 mg/d (0.3 mg/kg, Ulcer), up to 60 mg/d (1 mg/kg, Zollinger-Ellison).

- **Chronic toxicity studies**

Reversible hyperplasia of the gastric mucous membrane cells, thickening of the mucous membrane (dog 1 year study: 28 mg/kg, rat 6 months study: 138 mg/kg).

- **Carcinogenesis**

Mouse: negative results (max dose 138 mg/kg).
Rat: enterochromaffin cell hyperplasia and gastric tumors from 1.7 mg/kg in females.
Suspension of clinical trials.

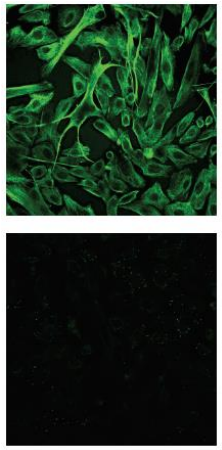
- **Is omeprazole a direct carcinogen?**

Mutagenesis tests: negative.
Inhibition of gastric acid secretion plasma gastrin trophic effects on gastric mucosa.
Treated patients: plasma gastrin x 1.3 to x 3.6

6 How to evaluate carcinogenic effects?

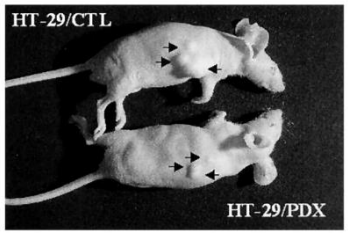
- **Experimental studies**

EMT

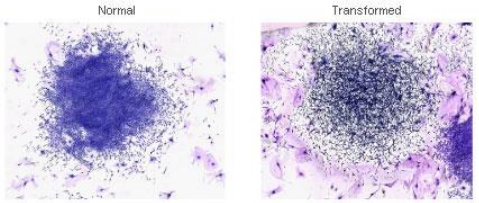


Soft agar clonogenic test

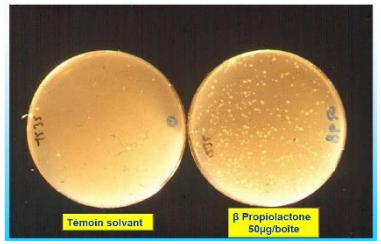
Tumor development in nude mice

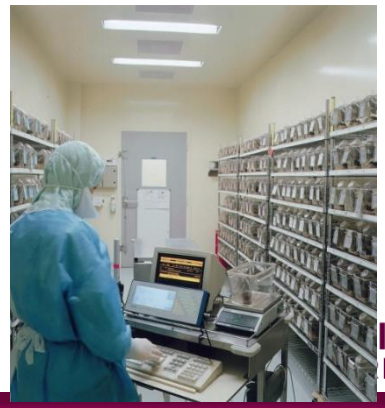


SHE test



- **Regulatory studies**





▶ **Genotoxic carcinogens**
Mutagenesis studies (regulatory battery)

▶ **Non-genotoxic carcinogens**
Carcinogenesis studies in rodents

6 How to evaluate carcinogenic effects?

Carcinogenicity studies in drug development

S1A-C

Long-term study in **rodents**

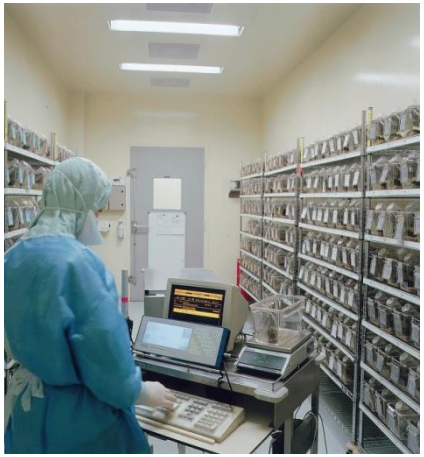
and

A complementary rodent study of shorter duration (e.g. transgenic models)

Or

A second carcinogenesis study in a second rodent species.

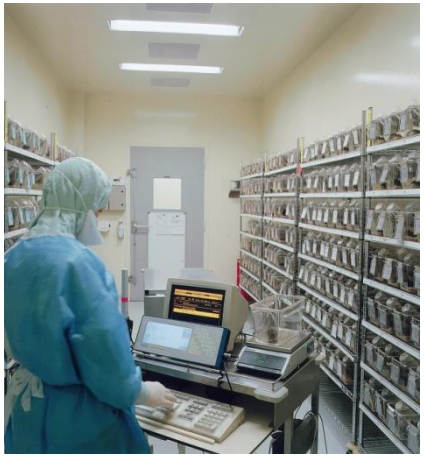
6 How to evaluate carcinogenic effects?
Carcinogenicity studies in drug development



The standard rodent protocol

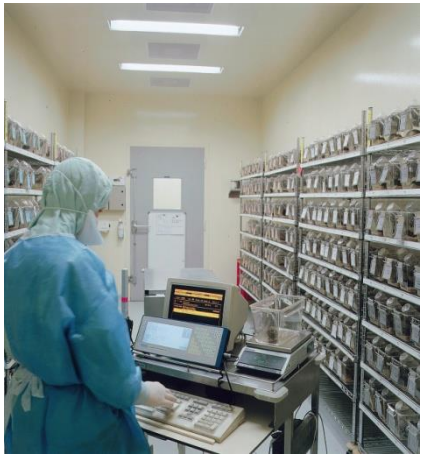
- **Species/strain:**
  - ⇨ Sprague-Dawley rats
  - ⇨ CD1 mice
- **Age:**
  - ⇨ At weaning
- **Duration of study:**
  - ⇨ 24 months (rat)
  - ⇨ 18 to 24 months (mice)
- **Route of administration**
  - ⇨ *Gavage or in food (same route of administration as in humans)*
- **Frequency**
  - ⇨ 7 days/week
- **Nb of groups**
  - ⇨ 1 or **2** controls, 3 doses
- **Nb of animals**
  - ⇨ ≥ 50/sex/group

6 How to evaluate carcinogenic effects?
Carcinogenicity studies in drug development



The standard rodent protocol

- **Clinical signs:** Daily (mortality); thorough, once a week
- **Behaviour:** Weekly
- **Palpable masses:** Monthly to weekly
- **Body weight:** Weekly
- **Histopathology:** All animals



The high dose

Defined as follows:

- Toxic but **not life-threatening**
- Causes a **decrease in weight gain ≤ 10%**
- Ensuring **exposure** ≥ 25 times the therapeutic (maximum) dose exposure

6 How to evaluate carcinogenic effects?

Carcinogenicity studies in drug development

PRODUCT CARCINOGENIC IF:

1. Presence of tumor types **not found in the control sample**

2. Increased tumor incidence compared to control sample (same tumor type)

3. **Early** tumor development compared to controls

6 How to evaluate carcinogenic effects?
Carcinogenicity studies in drug development

Oral Carcinogenicity Study in Rats

Document Control Number:							**Test Article:**					
Daily Dose (mg/kg)	**(0) Control**		**0 (Control)**		**0.003**	**0.01**	**0.02**	**0.06**	**0.2**	**0.4**	**1.4**	**2.6**
Number of Animals	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F: 60**	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F:60**
Food Consumption (%[b]) (g/kg/day)	46	58	+2	0	+4**	+2	+7**	+3	+7**	+7**	+9**	+9**
Histopathology - Neoplastic Lesions[c]												
Brain, malignant glioma	0	0	0	0	1	0	1	1	2	0	4 #	3 #
malignant oligodendroglioma	0	0	0	0	0	0	0	0	0	0	0	1
Kidney, oncocytoma	0	0	0	0	0	0	0	0	0	0	2 #	0
malignant mesenchymal tumor	0	0	0	0	0	0	0	0	0	0	2 #	0
Liver, hepatocellular adenoma	0	0	0	1	0 (59)	2 (57)	1	3	0	1	1	8 #
hepatocellular carcinoma	0	0	0	0	0 (59)	0 (57)	1	0	0	0	0	3 #
Pancreas, acinar adenoma	0 (59)	0	0 (58)	0	0 (59)	0	0 (59)	0	2	0	2	0
acinar carcinoma	0 (59)	0	0 (58)	0	0 (59)	0	0 (59)	0	0	0	1	0
mixed islet-acinar cell neoplasm	0 (59)	0	0 (58)	0	0 (59)	0	0 (59)	0	0	0	1	0
Skin, fibroma	1 (59)	0	1	0	0	0	1	1	2 (59)	2 (57) #	1 (59)	3 #

Abbreviations: - No noteworthy findings; ♦ - Not evaluated.
Statistical Analysis: * <0.05 ** - p<0.01 Dunnett's Test; # - Denotes statistical significance by Peto analysis.

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Carcinogenicity studies in drug development

Oral Carcinogenicity Study in Mice

Document Control Number:							Test Article:					
Daily Dose (mg/kg)	**(0) Control**		**0 (Control)**		**0.004**		**0.04**		**0.4**		**4.0**	
Number evaluated	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F:60**	**M:60**	**F:60**
Histopathology - Neoplastic Lesions [c]												
Liver												
hepatocellular adenoma	8	0	4	2	4	0	2	1	8	0	7	2
hepatocellular carcinoma	1	0	0	0	1	0	3	1	2	0	8 [#]	0
Lung												
bronchioloalveolar adenoma	4	9	4	6	8	5	11 (58) [#]	4	17 [#]	16	20 [#]	15 [#]
bronchioloalveolar carcinoma	3	3	3	5	4	3	4 (58)	2	7	5	15 [#]	16 [#]
combined bronchioloalveolar tumors	7	12	7	11	12	8	15 (58) [#]	6	24 [#]	21 [d]	35 [#,d]	31 [#,d]
Ovary												
hemangioma	♦	3	♦	3 (58)	♦	6 (59)	♦	7	♦	5	♦	19 [#]
Parotid salivary gland												
ductal adenoacanthoma	0 (59)	0	0	0	0	0 (59)	0 (59)	0 (59)	0	0	0	2 (58)

Abbreviations: ♦ - Not evaluated.
Statistical Analysis: * - P<0.05 ** - P<0.01 Dunnett's Test; [#] - Denotes statistical significance by Peto analysis.
All footnotes are available as table end notes.