# Preventing Cancer — How does it Work?

Diagnosis and Treatment of Vitamin D Deficiency Symposium
University of California San Diego


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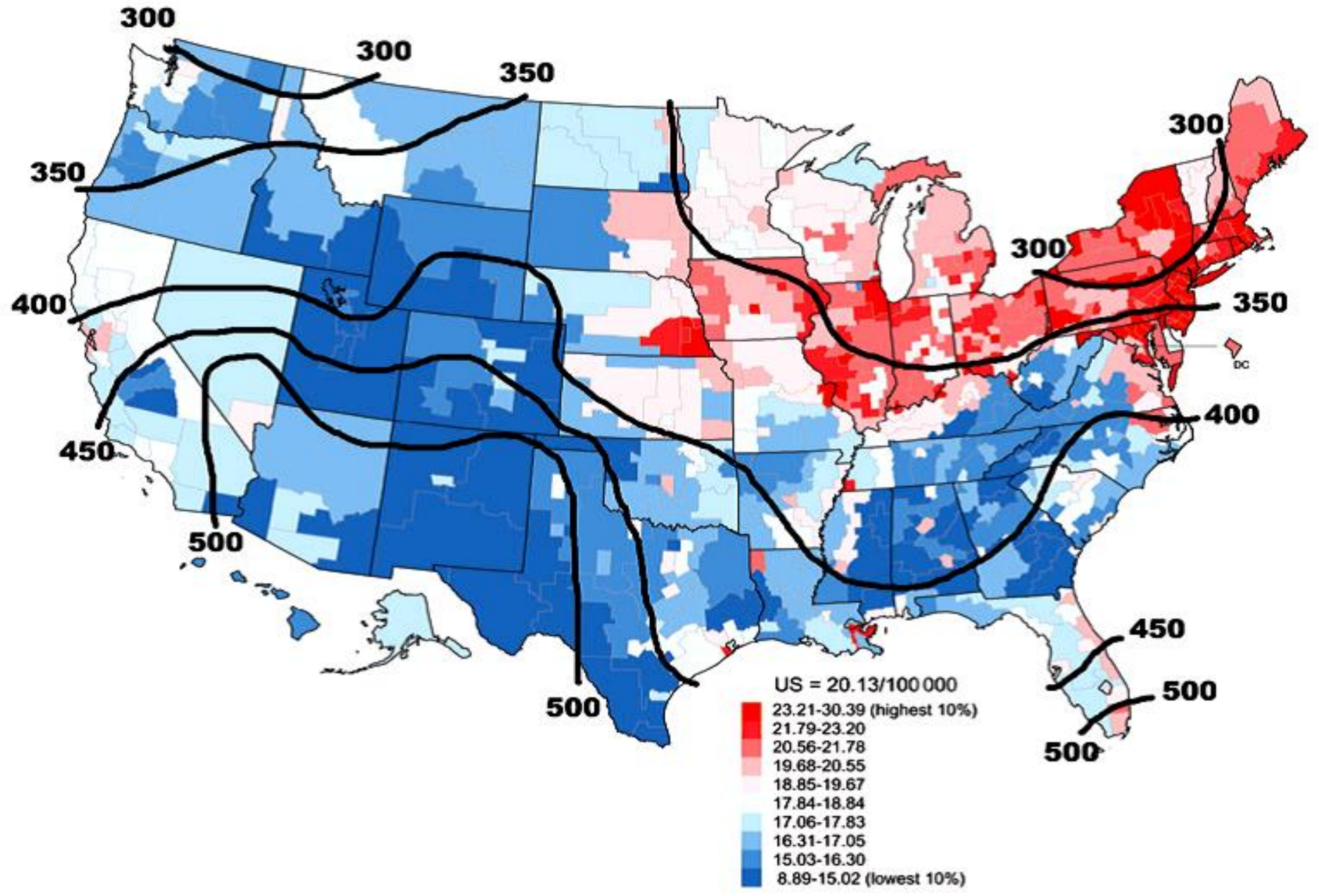
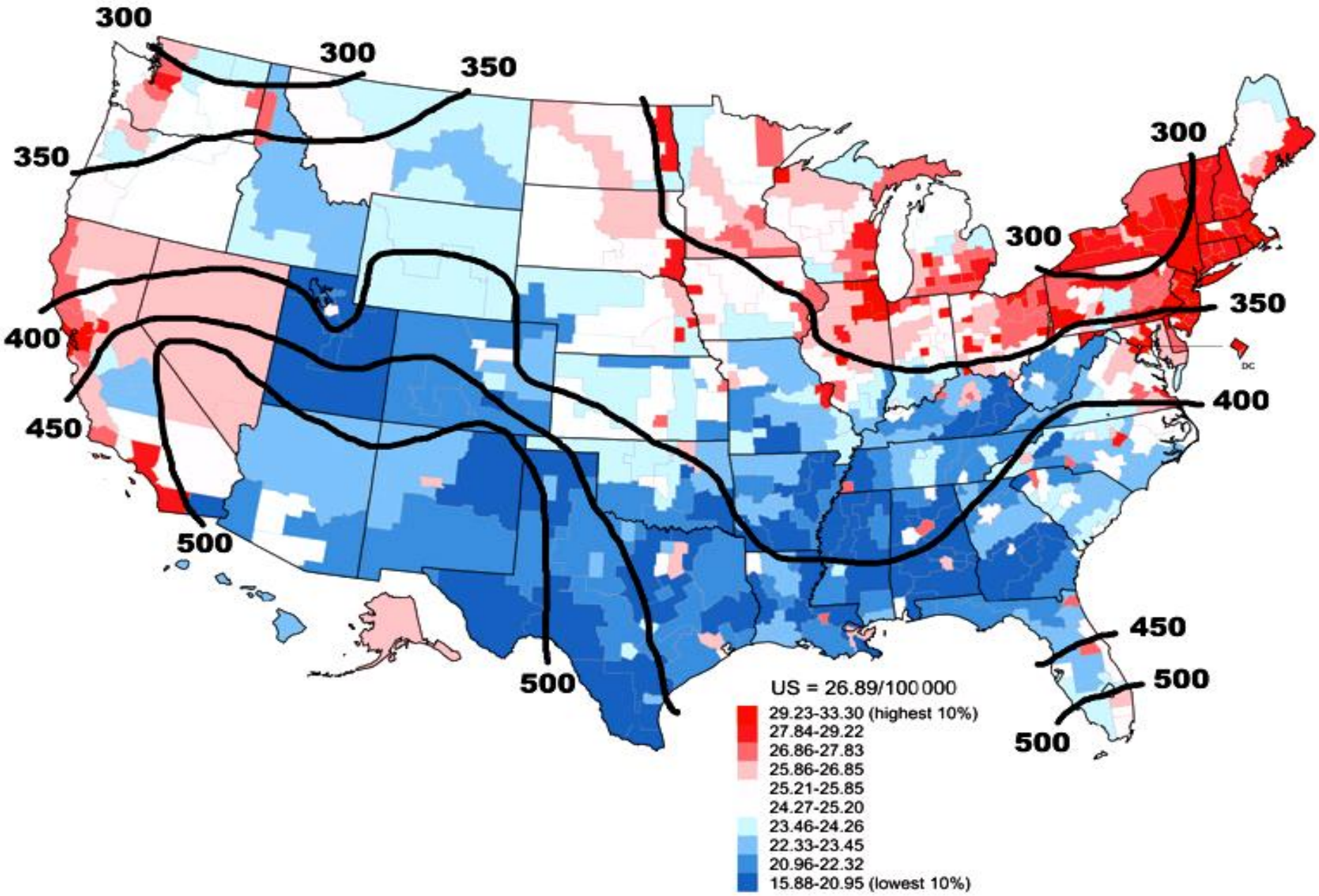
# Disclosure

- I have no actual or potential conflict of interest in relation to this program.
- Sources are cited at the conclusion of this presentation.

# Colon Cancer Mortality Rates, USA



# Breast Cancer Mortality Rates, USA



# Truth…

**"All truth passes through three stages.**

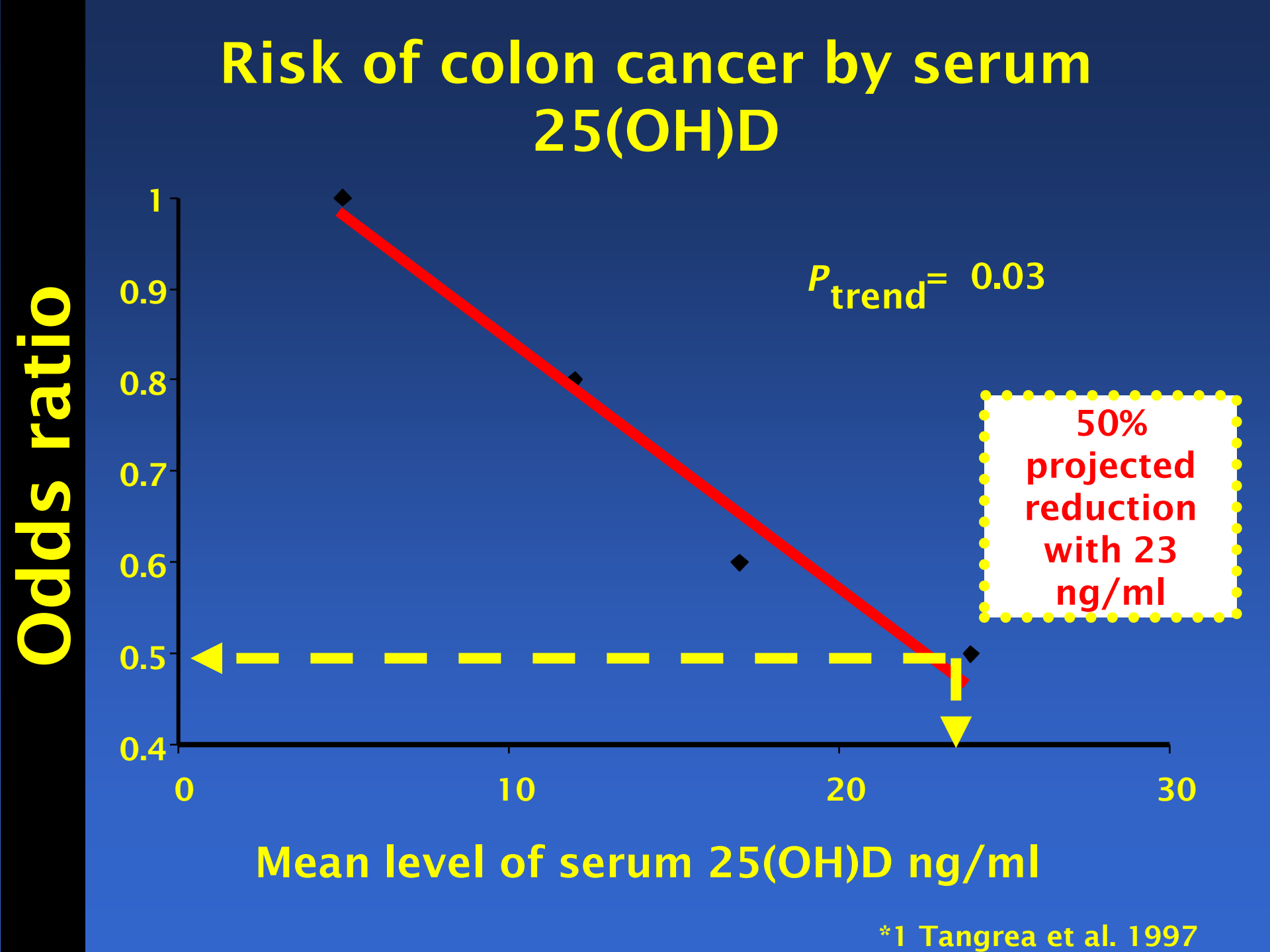
**First:** it is *ridiculed*.

**Second:** it is violently *opposed*.

**Third:** it is accepted as being *self-evident*."

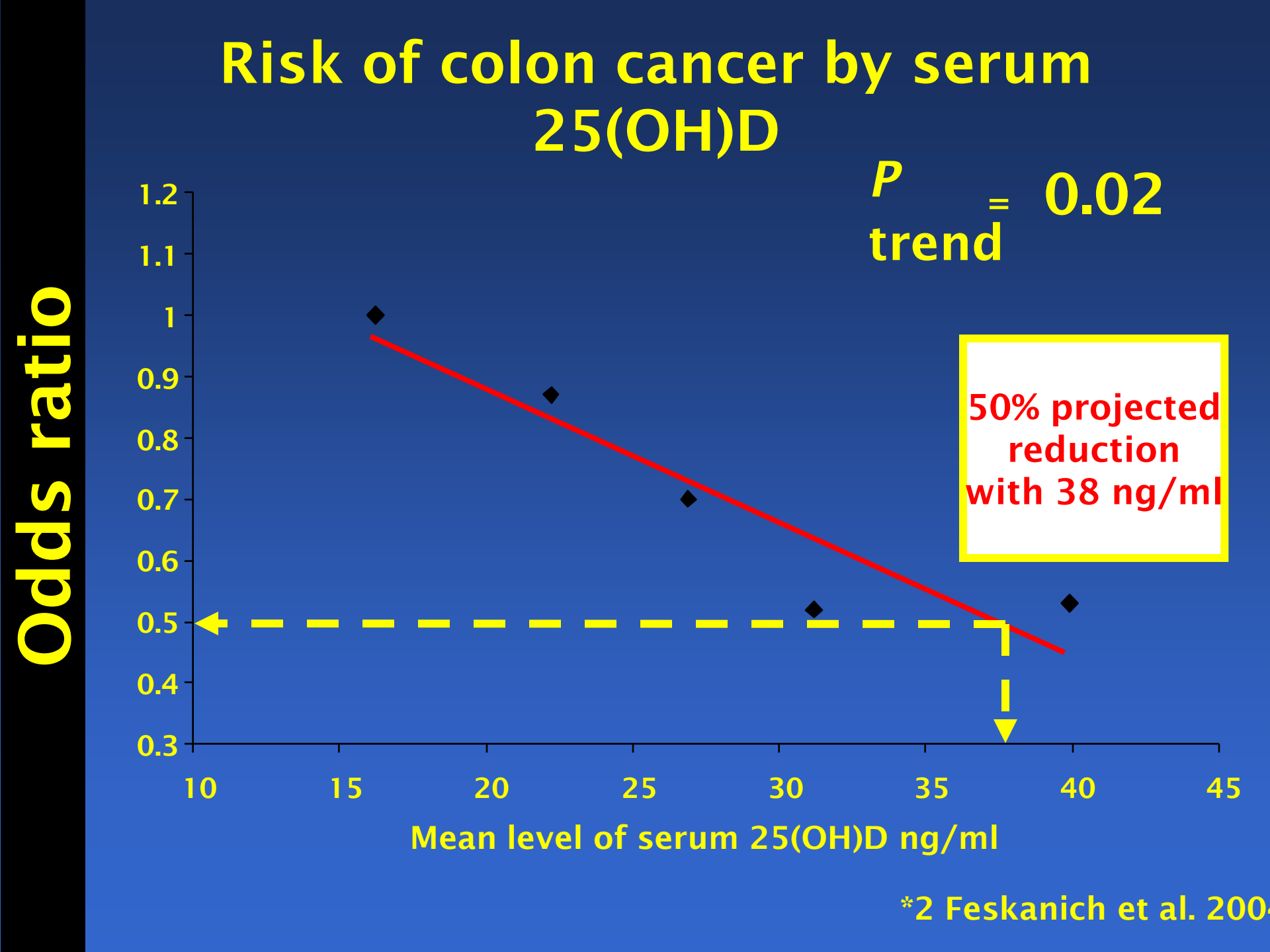
-Schopenhauer

# Risk of colon cancer by serum 25(OH)D



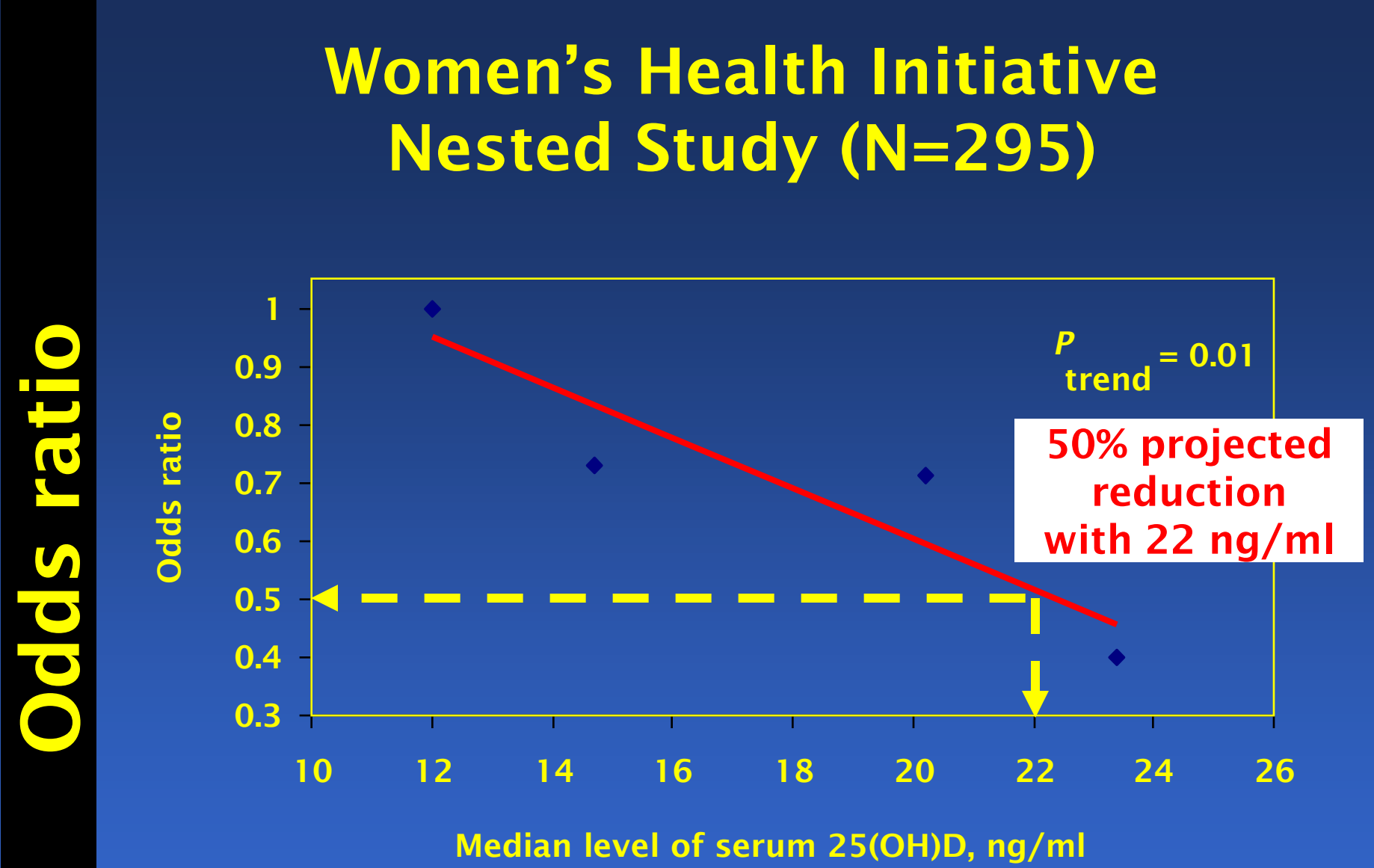

*1 Tangrea et al. 1997

# Risk of colon cancer by serum 25(OH)D



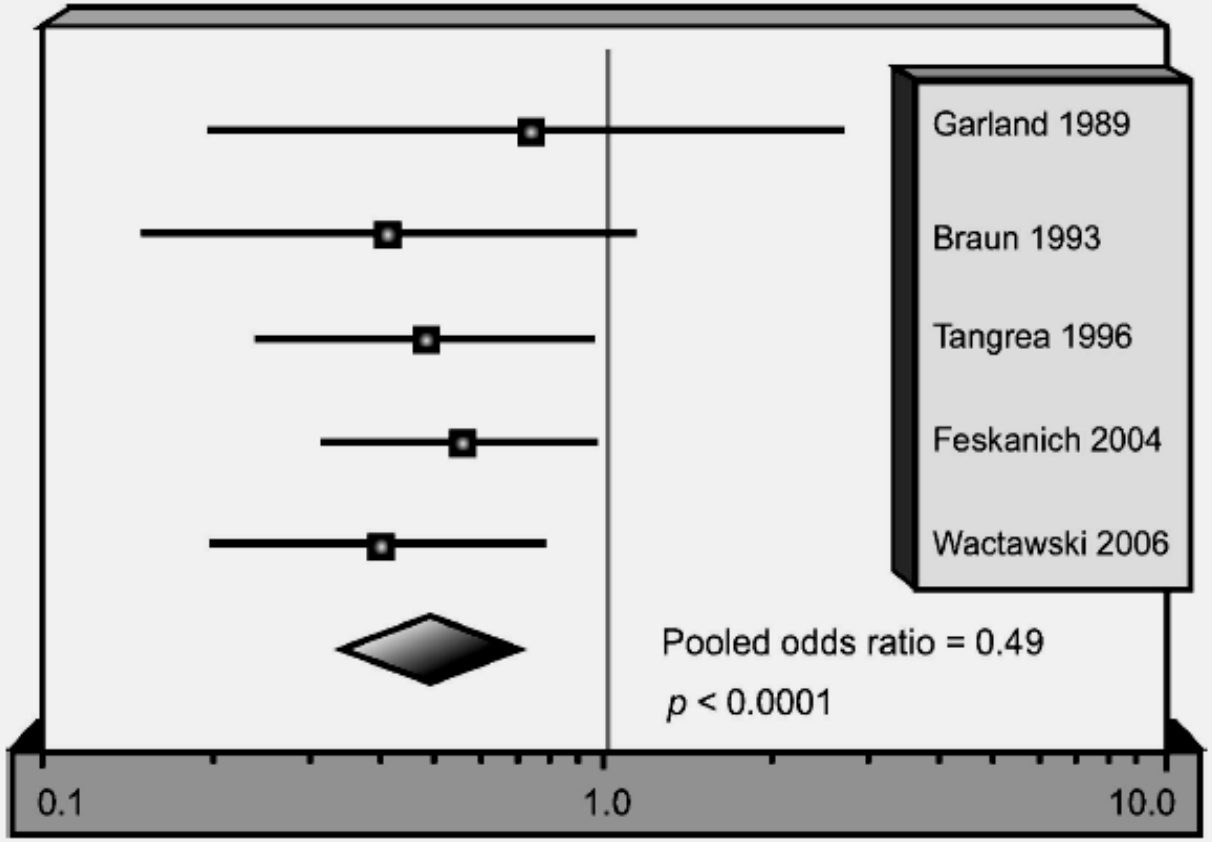

*2 Feskanich et al. 200

# Women's Health Initiative Nested Study (N=295)




*3 Wactawski-Wende

# Meta-analysis




Forest plot of all studies of serum 25(OH)D and risk of colorectal cancer. *4 The upper and lower 95% confidence limits on the odds ratio are denoted by horizontal lines for each study, and the 95% confidence limits for the combined estimate for all studies are denoted by the points of the diamond. The odds ratios compare the highest quintile to the lowest.

# Meta-analysis

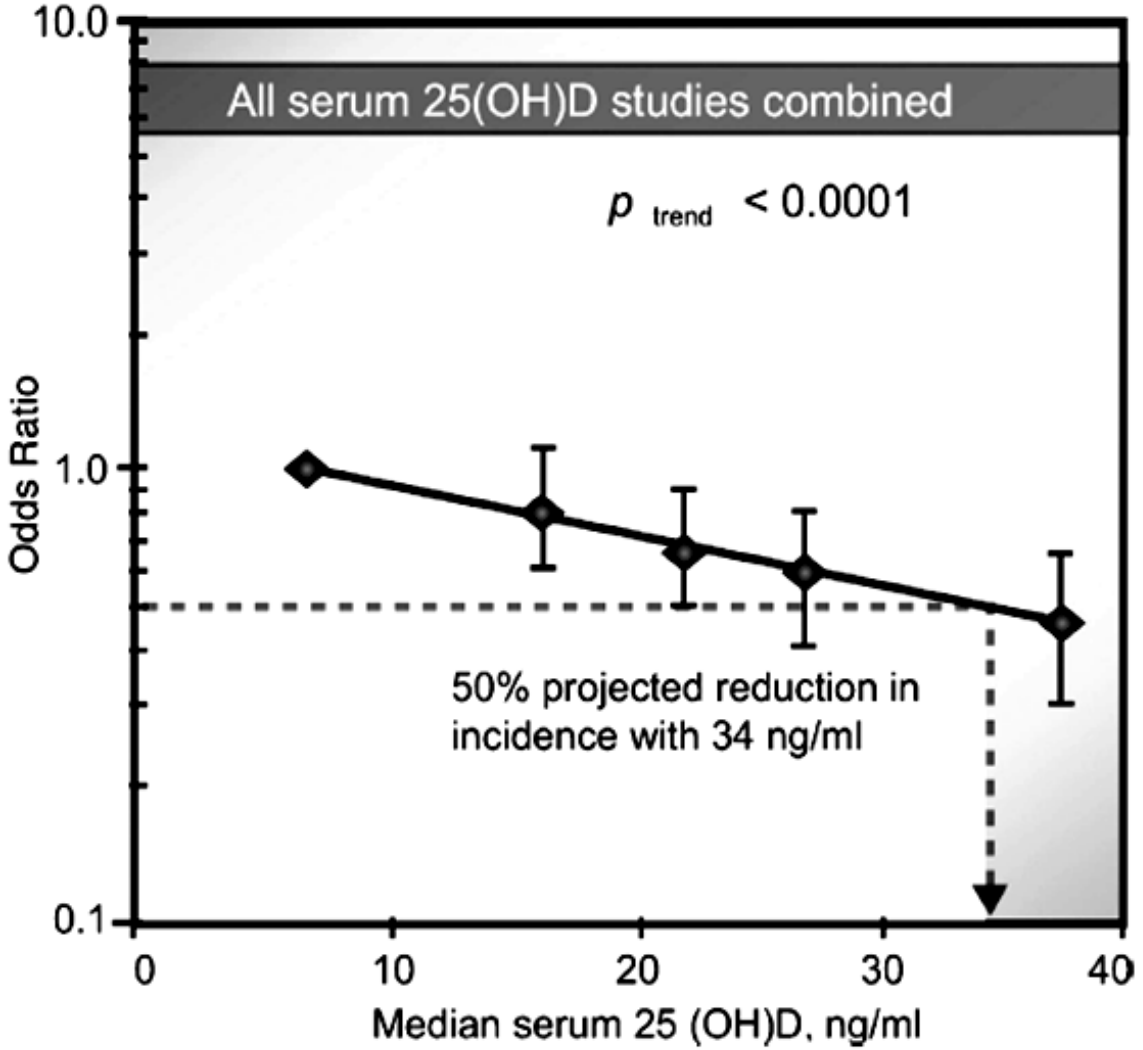
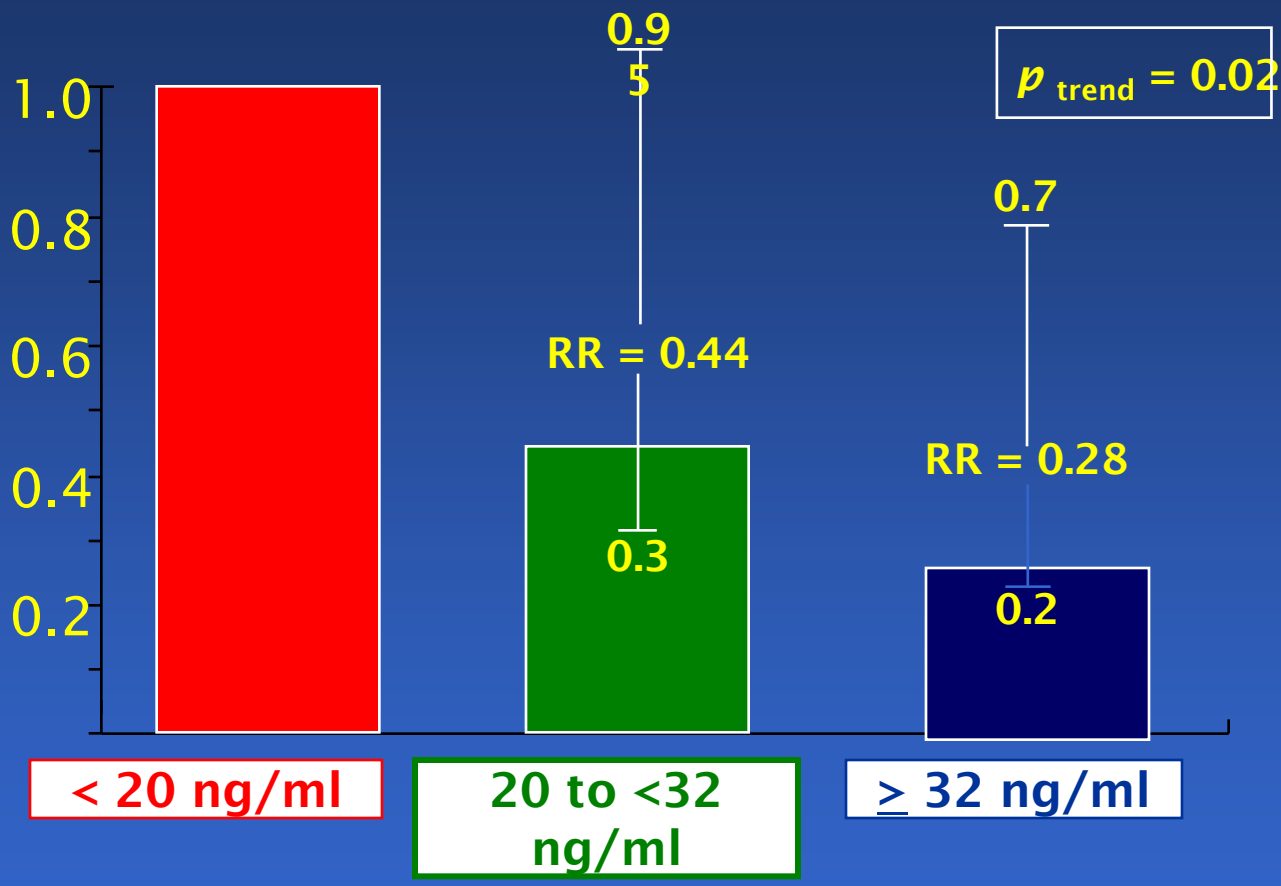


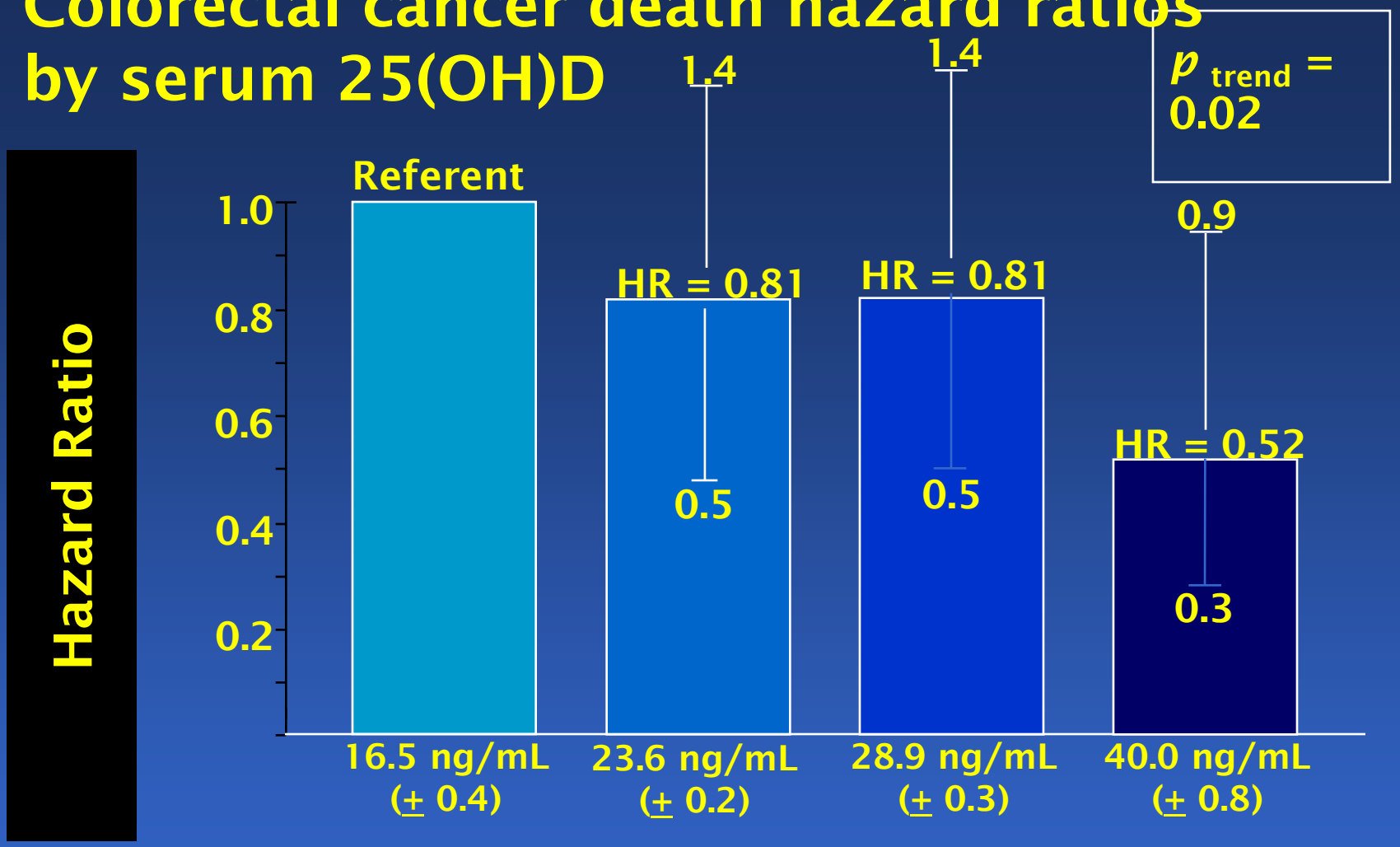

**Figure 1.** Dose–response gradient for colorectal cancer according to serum 25(OH)D concentration, all five studies combined.[1,4–7] The five points are the odds ratios for each quintile of 25(OH)D based on combined data from the five studies.

# Colon cancer mortality risk by prediagnostic serum 25(OH)D in the USA



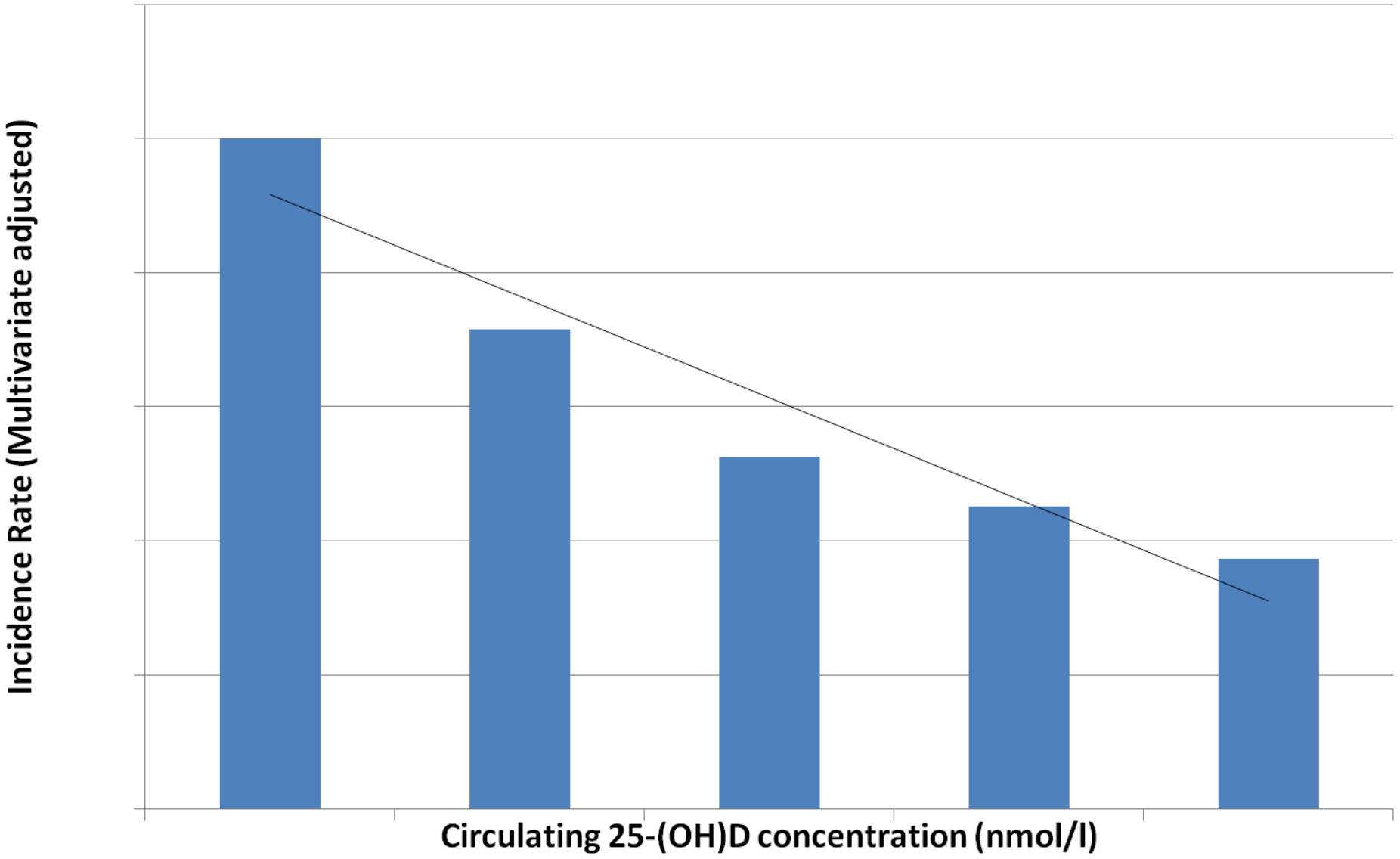

Relative risk of colon cancer mortality, by baseline serum 25-hydroxyvitamin D concentration tertiles, NHANES III cohort, 1988-2000

# Colorectal cancer death hazard ratios by serum 25(OH)D

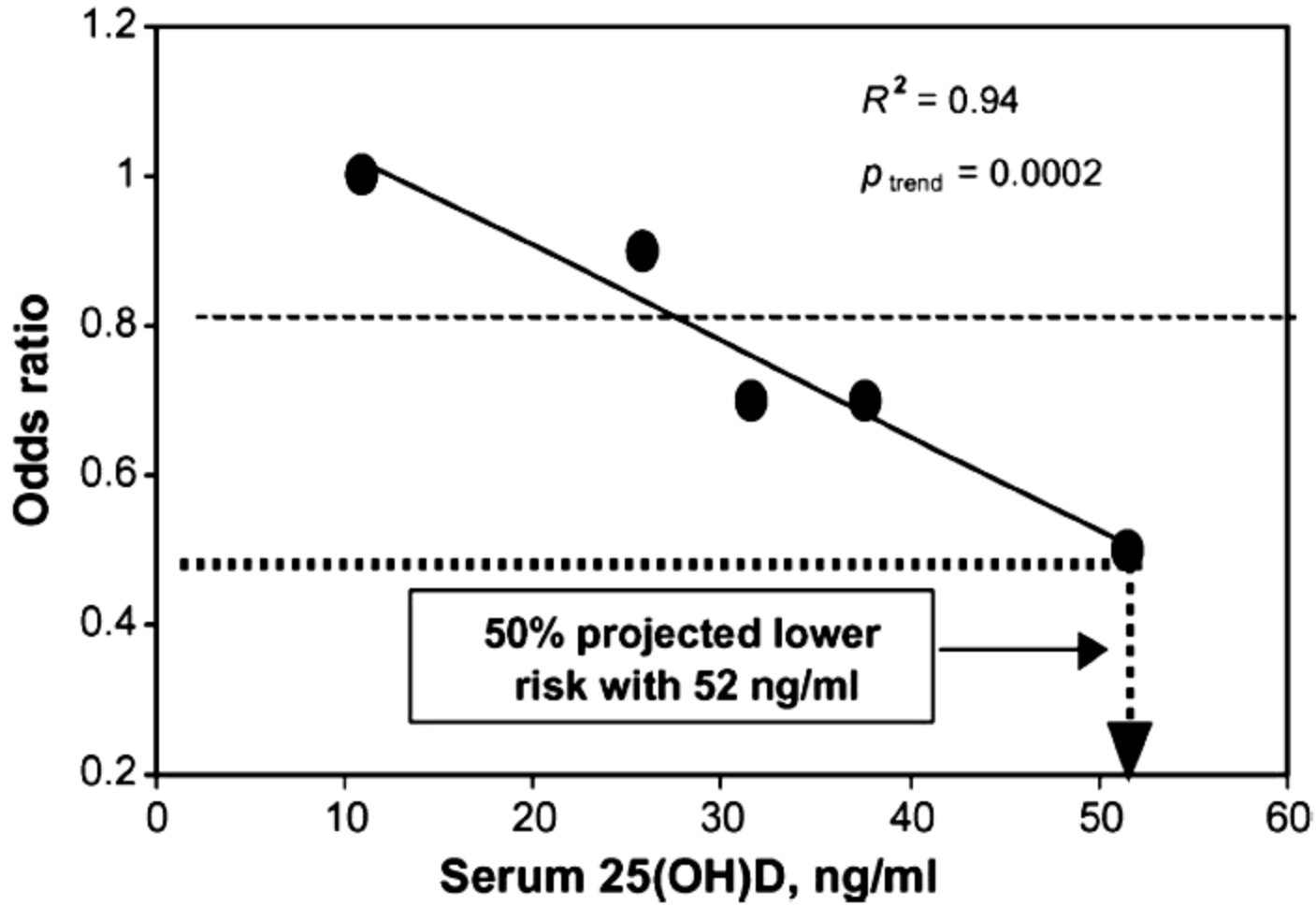



Multivariate-adjusted hazard ratios for death, 304 colorectal cancer patients, by prediagnostic mean plasma 25-hydroxyvitamin D concentration quartiles, Nurses Health and Health Professionals Study Cohorts * 6

# Colon Cancer Incidence By Circulating 25 OH D (EPIC study)



# Meta-analysis of breast cancer risk




Dose–response gradient of risk of breast cancer according to serum 25-hydroxyvitamin D concentration, pooled analysis.

# Breast cancer risk by 25(OH)D

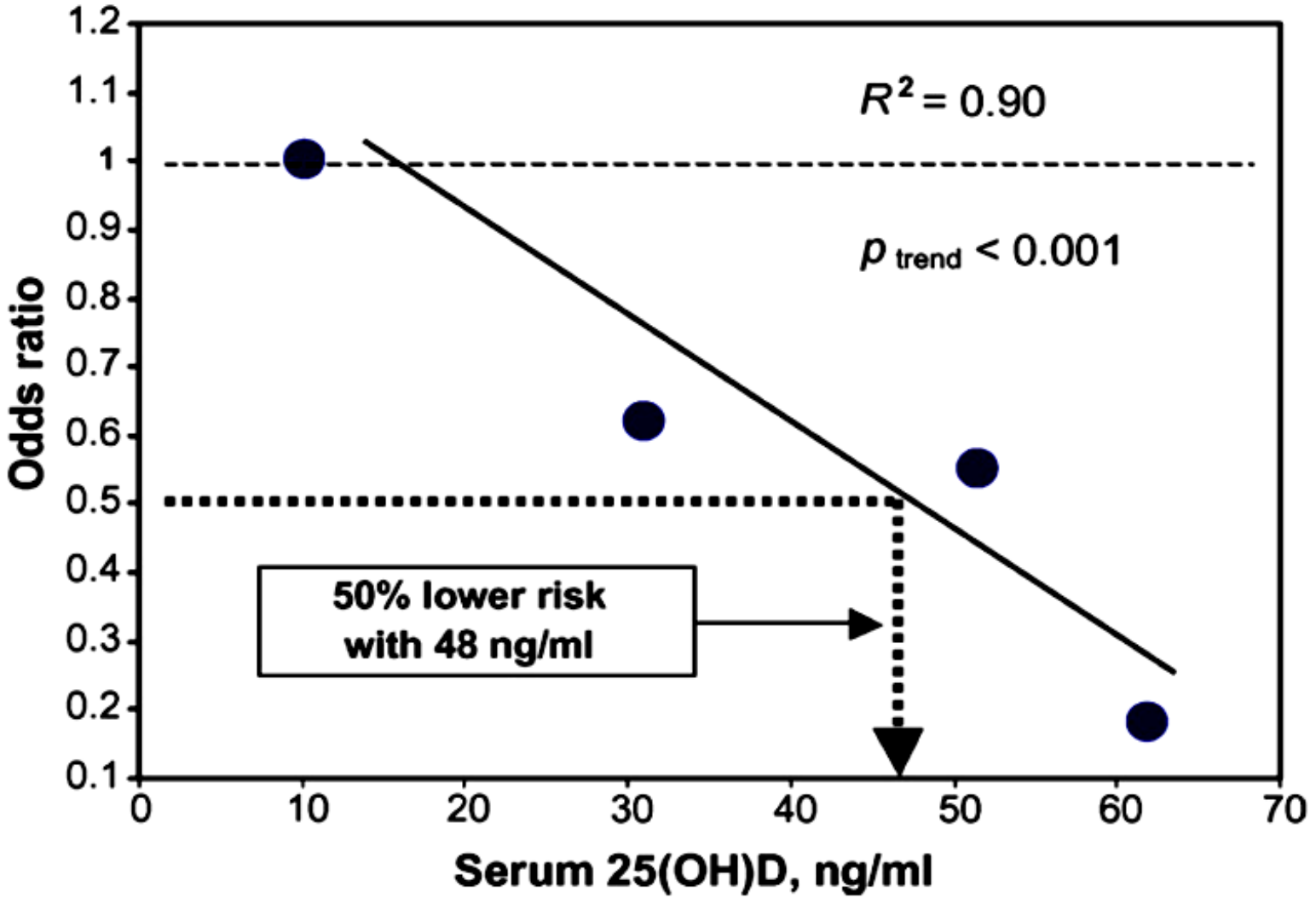
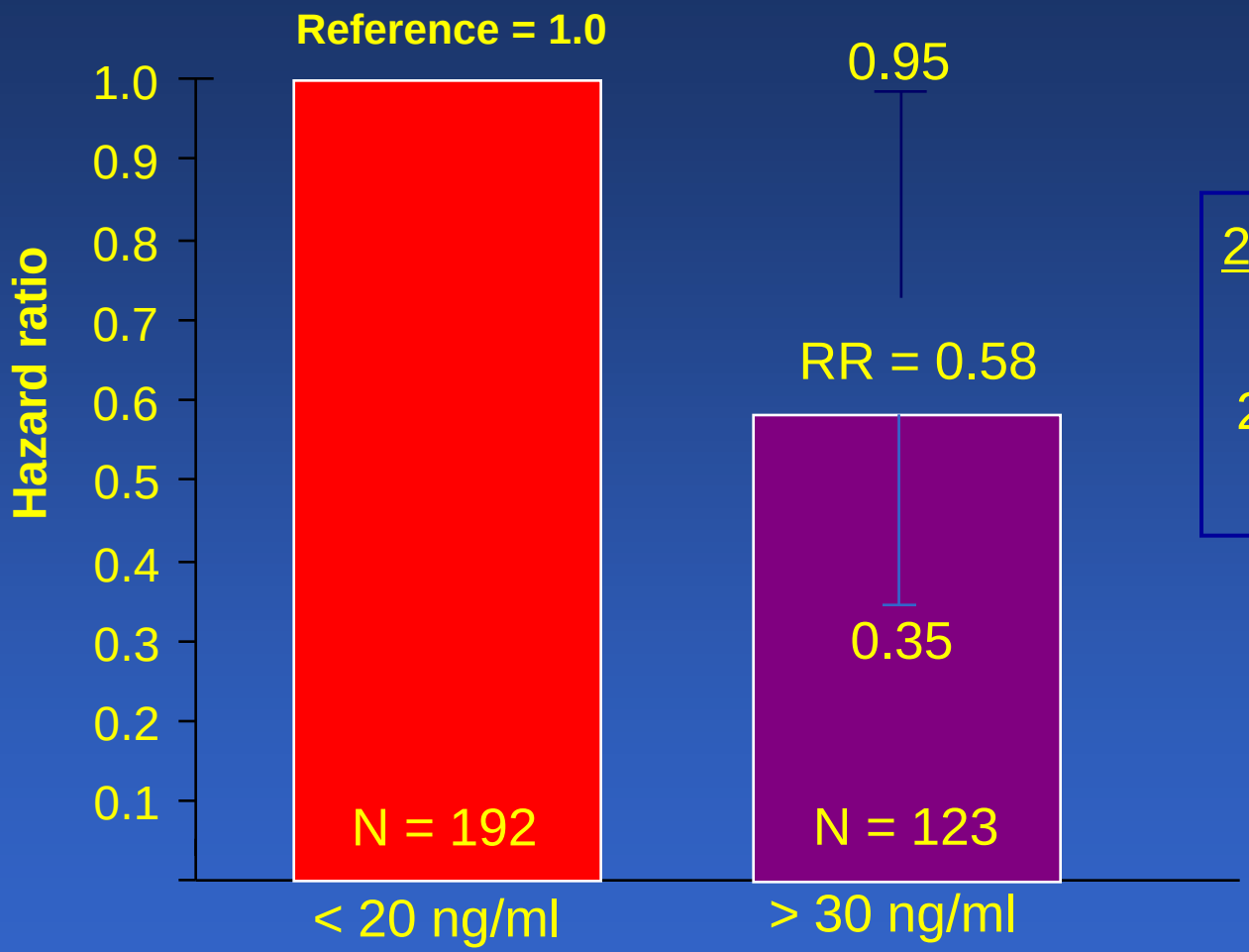



Fig. 2. Dose–response gradient of risk of breast cancer according to serum 25-hydroxyvitamin D concentration, St. George's Hospital, London

# Hazard of death, 512 women with breast cancer, by 25(OH)D level at diagnosis, median follow-up 11.6 years, Toronto, Canada




*p* = 0.02

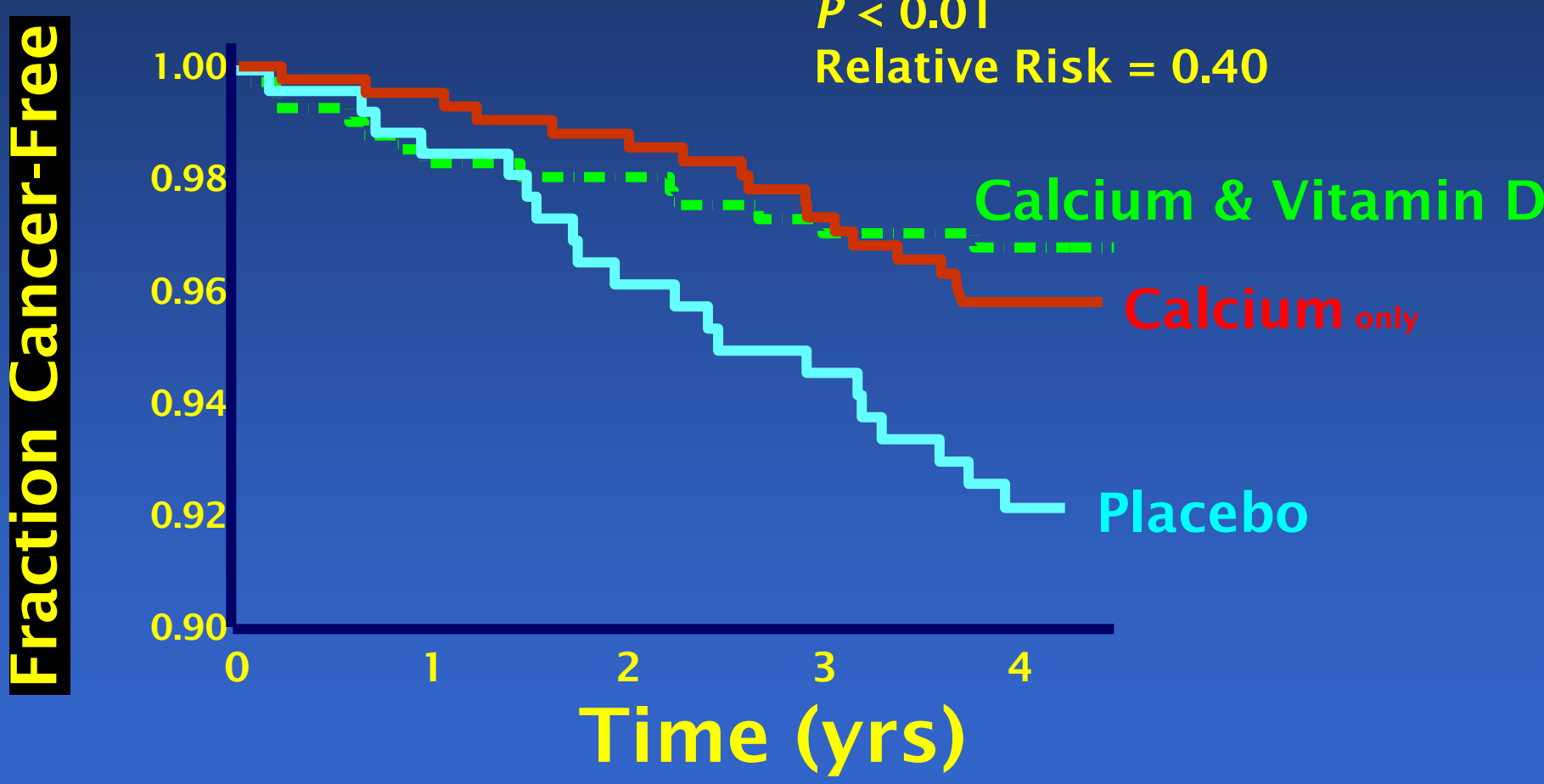
| 25 (OH)D at dx (ng/ml) | |
|---|---|
| < 20 | N= 192 |
| 20 - 29.9 | N= 197 |
| > 30 | N= 123 |

Hazard ratio and 95% confidence intervals for overall survival by 25(OH)D serum level at diagnosis, Toronto, Canada (latitude $43^0$ 40 N')

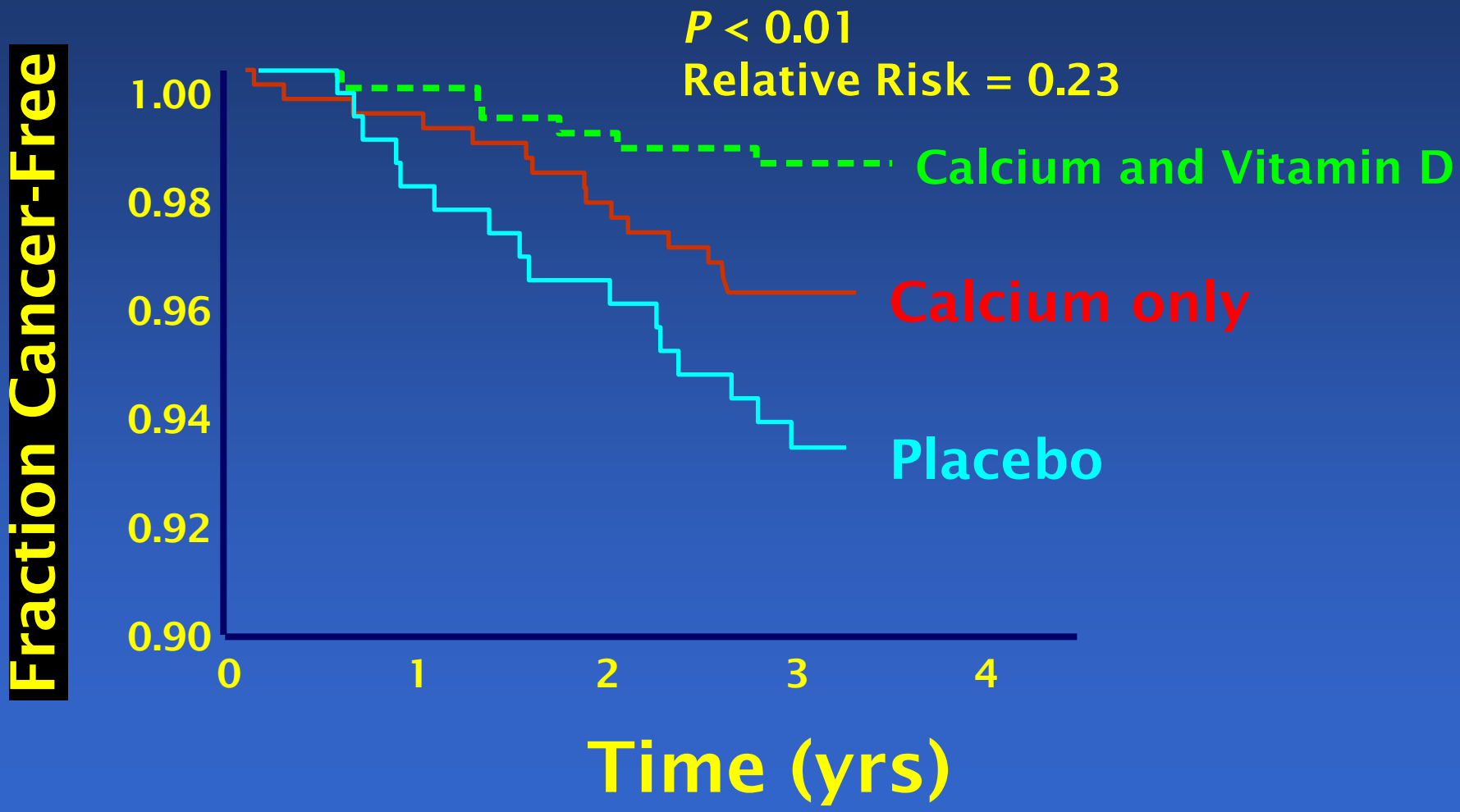
# Lappe et al. Randomized Controlled Trial of Vitamin D and Calcium in Cancer (2007)

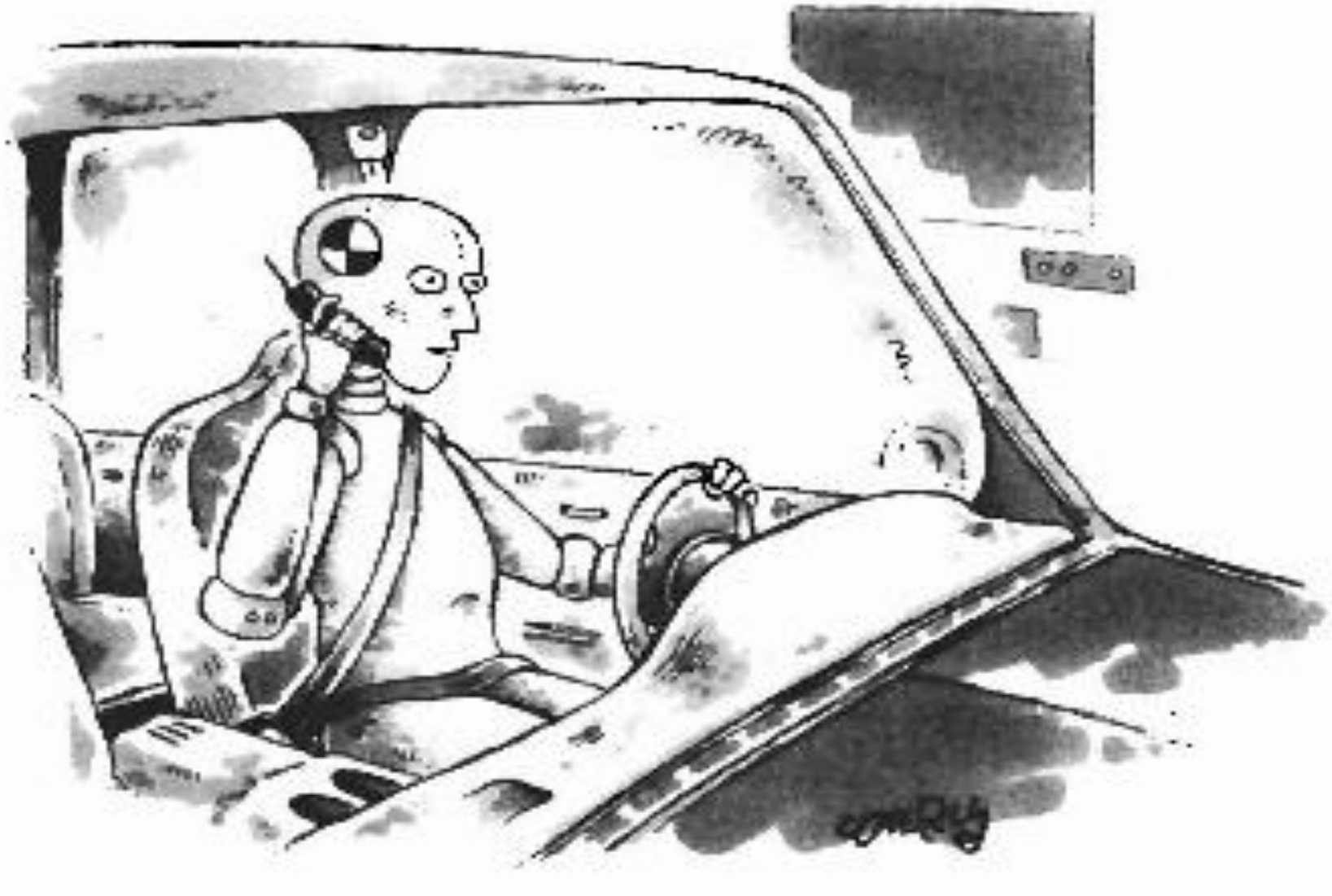
- Four years, N = 1,179 healthy women in Omaha NE
- Mean age 66.7 ± 7.3 years
- N = 1,032 finished trial (87.5%)
- Baseline serum 25(OH)D: 29 ± 8 ng/ml (72 ± 20 nmol/L)
- Three treatment groups:
  - Vitamin $D_3$ (1,100 IU/day) and calcium (1450 mg/day)
  - Calcium (1,450 mg/day)
  - Placebo
- Outcome: All cancers except minor skin (mainly breast, lung and colon)

# Randomized Controlled Trial



# All Except First Year Cases



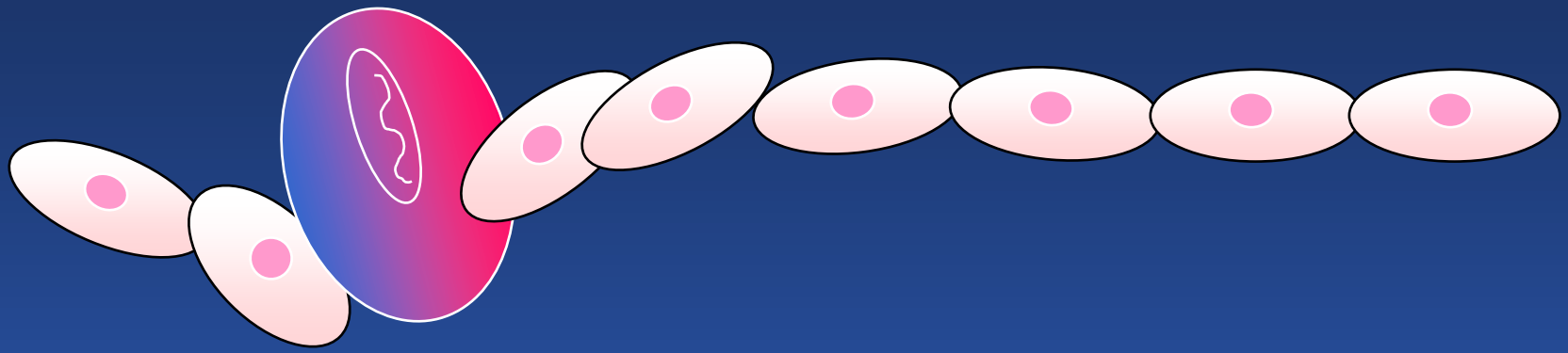


Well, I'd better go now. I'm almost at the wall.

# Epidemiological studies reporting no or adverse associations with serum 25(OH)D

- Stolzenberg-Solomon RZ et al. – Finland - Pancreatic cancer in male smokers, 50-69 yrs in ATBC study, particularly in winter months (*Cancer Res 2006*;66:10213-9)(Pickled herring vs. ?)
- Abnet C et al.– China - Esophageal squamous cell dysplasia/cancer in poor rural Linxian men but not women (*Br J Cancer* 2007;97:123-8 (Plant/mushroom source of vitamin D vs. malnutrition vs.?)
- Ahn et al. – PLCO - No association with prostate cancer incidence, but cases found in screening study were more advanced (*JNCI* 2008;100:796-804)(Possibly unmasking bias?; opposite result in Harvard HPFS.)
- Freedman et al. – PLCO -No association with breast cancer in nested case-control study (*Cancer Epidemiol Biomark Prev* 2008; 17:889-94)(Matching, latency issues)
- No association with breast cancer (Hiatt RA et al., JNCI 1998;90:461-3); 1,25 only with breast cancer (Janowsky et al. *Pub Health Nutr* 1999;2:283-91); Chlebowski RT et al. (*JNCI* 2008;100:1581-91) using 400 IU WHI trial, but favorable association with baseline 25(OH)D.

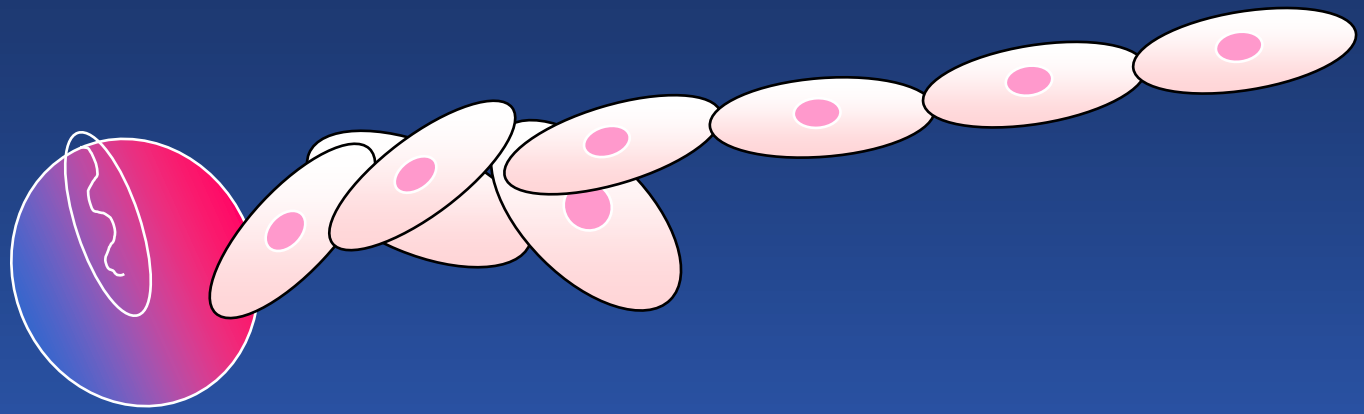
# Classical Theories of Carcinogenesis



## Mutation theory: Boveri, 1902

- Two-hit theory: Knudson, 1980.
- "Many-hit" theory: A number of hits are needed (authors include Vogelstein et al., 1991).

# Micro-Darwinian carcinogenesis and Vitamin D deficiency induced D-volution



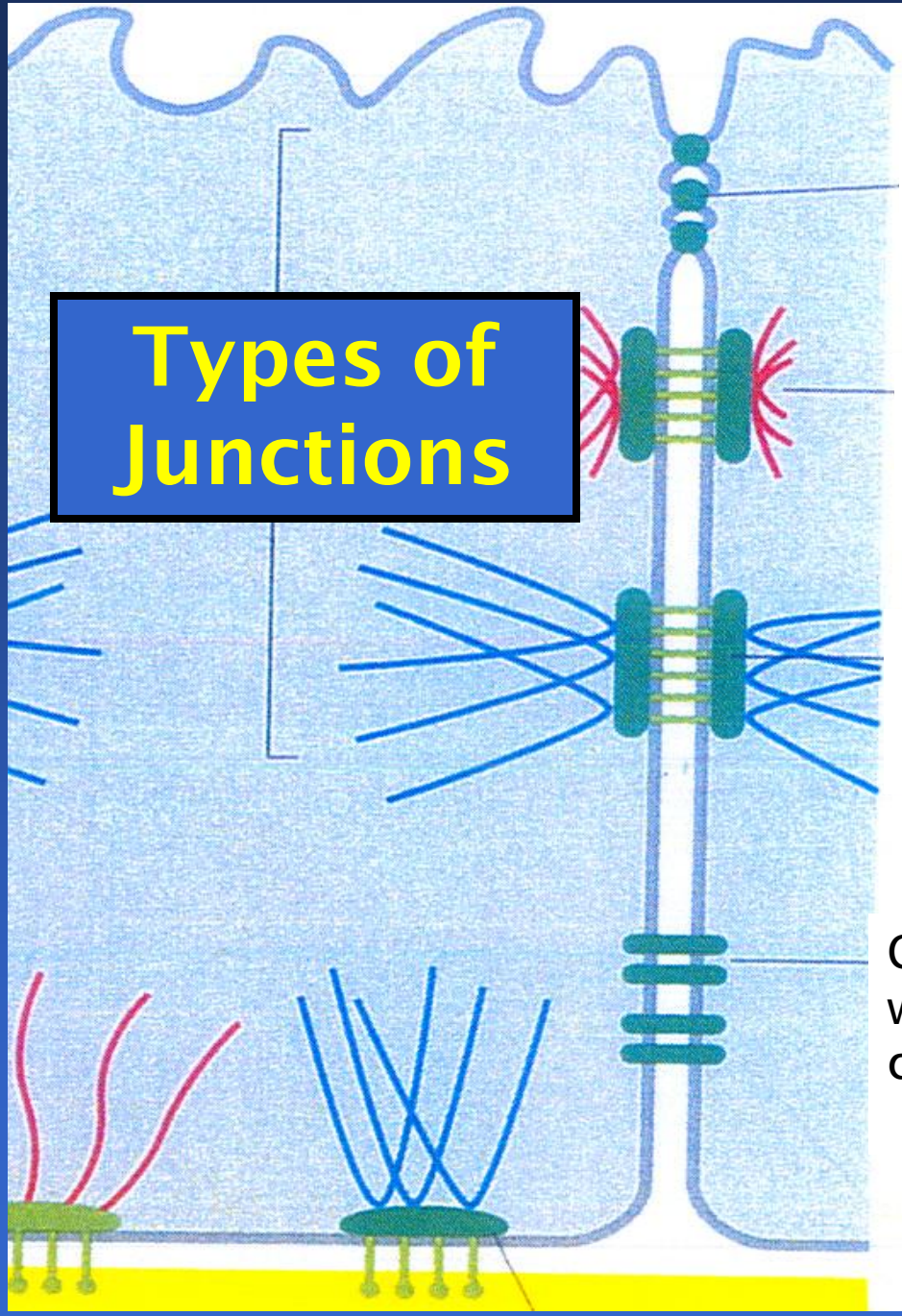
In vitamin D deficiency, the first lesion is harm to the intercellular junction.

This unleashes natural selection.
Natural selection is the engine of growth of the cancer.

# DINOMIT - Theory of Breast Cancer

- Disjunction – Loss of Tight Junctions
- Initiation – Genetic variation
- Natural selection – Competition for growth
- Overgrowth – Palpable mass and invasion
- Metastasis – Remote colonization
- Involution – Growth inhibition
- Transition – Coexistence with normal tissue

# Types of Junctions



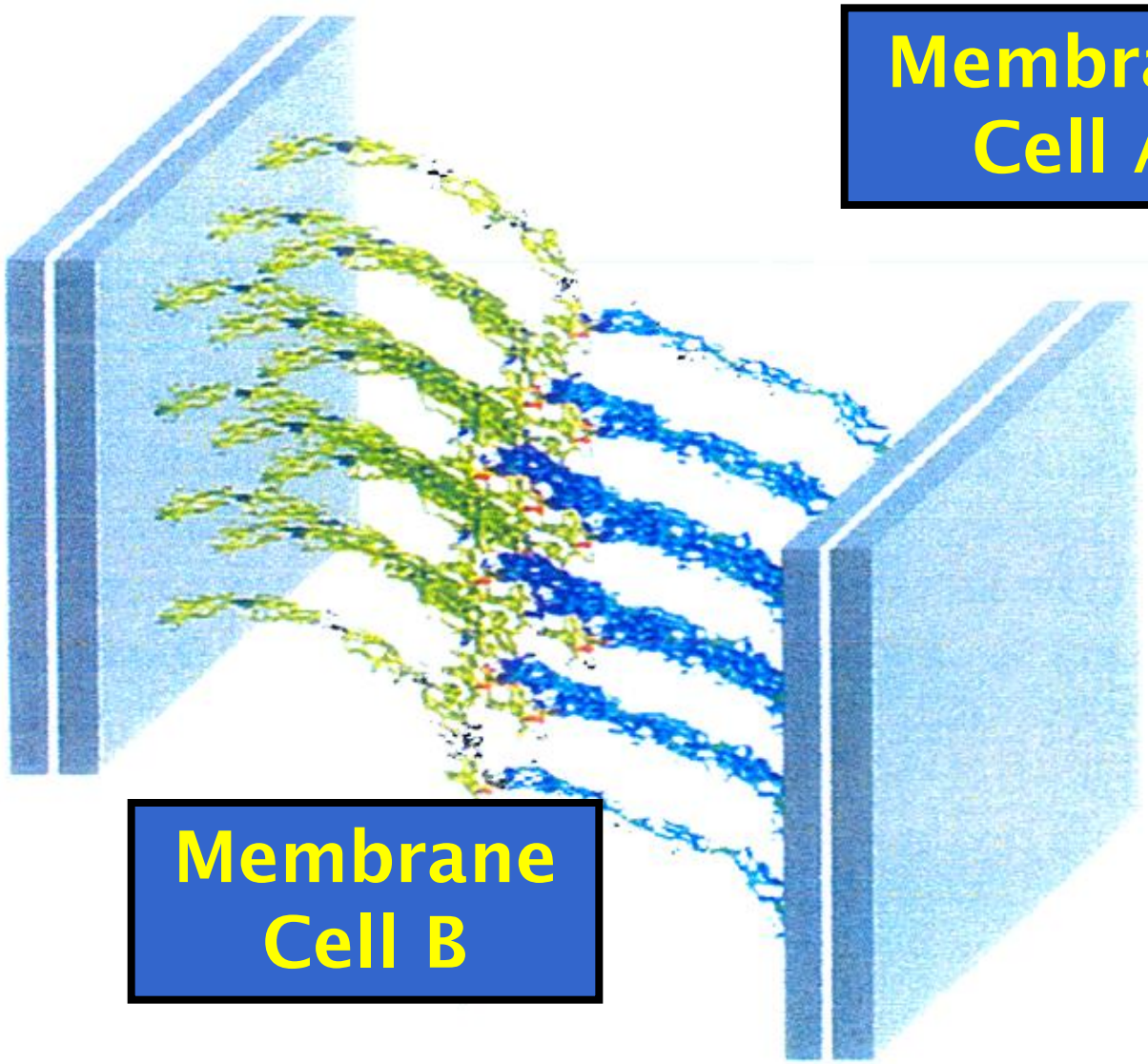
Tight junctions seal gaps between epithelial cells

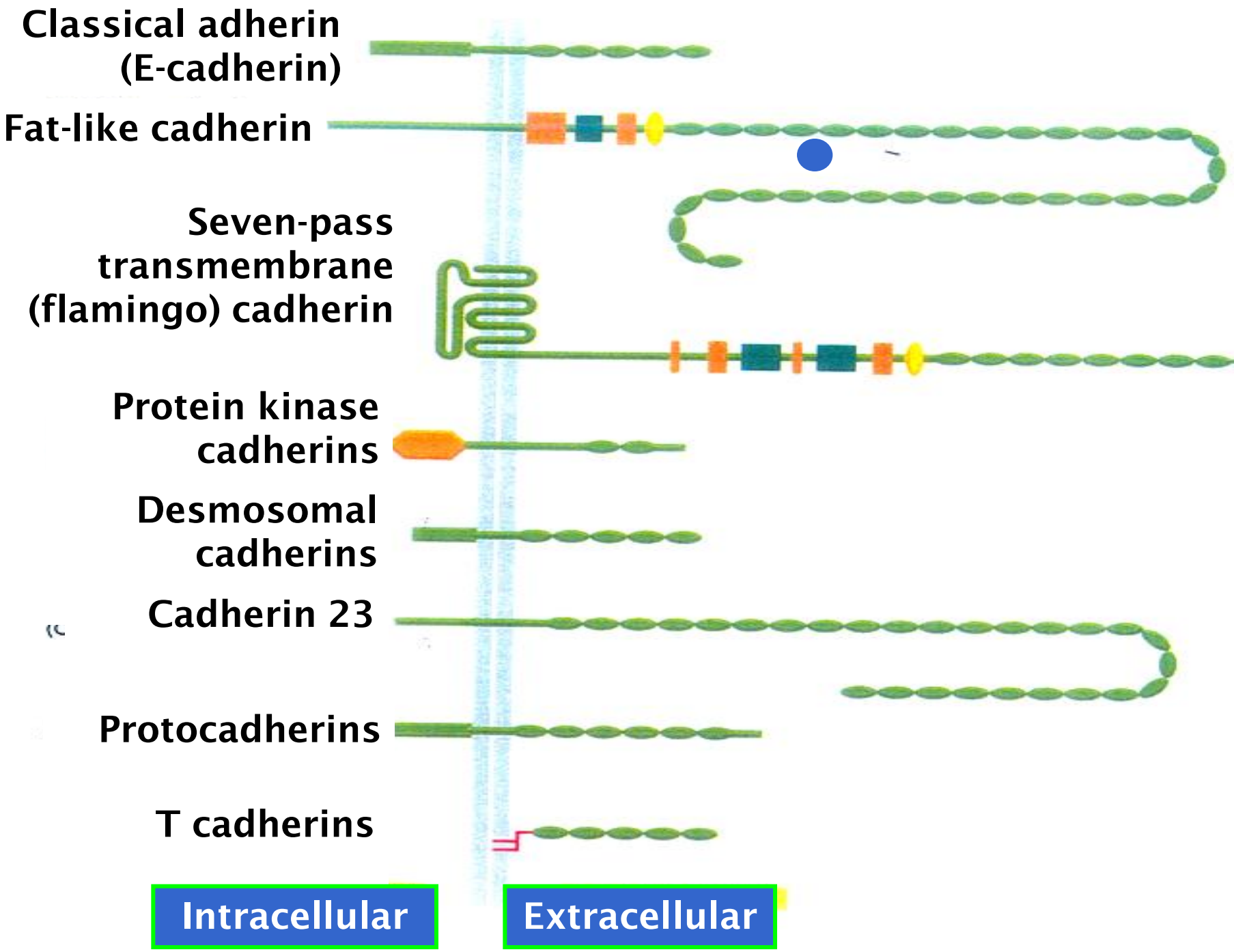
Adherens junctions connect actin filament bundles between cells

Desmosomes connect intermediate filaments in adjacent cells

Gap junctions allow passage of small water-soluble molecules between cells

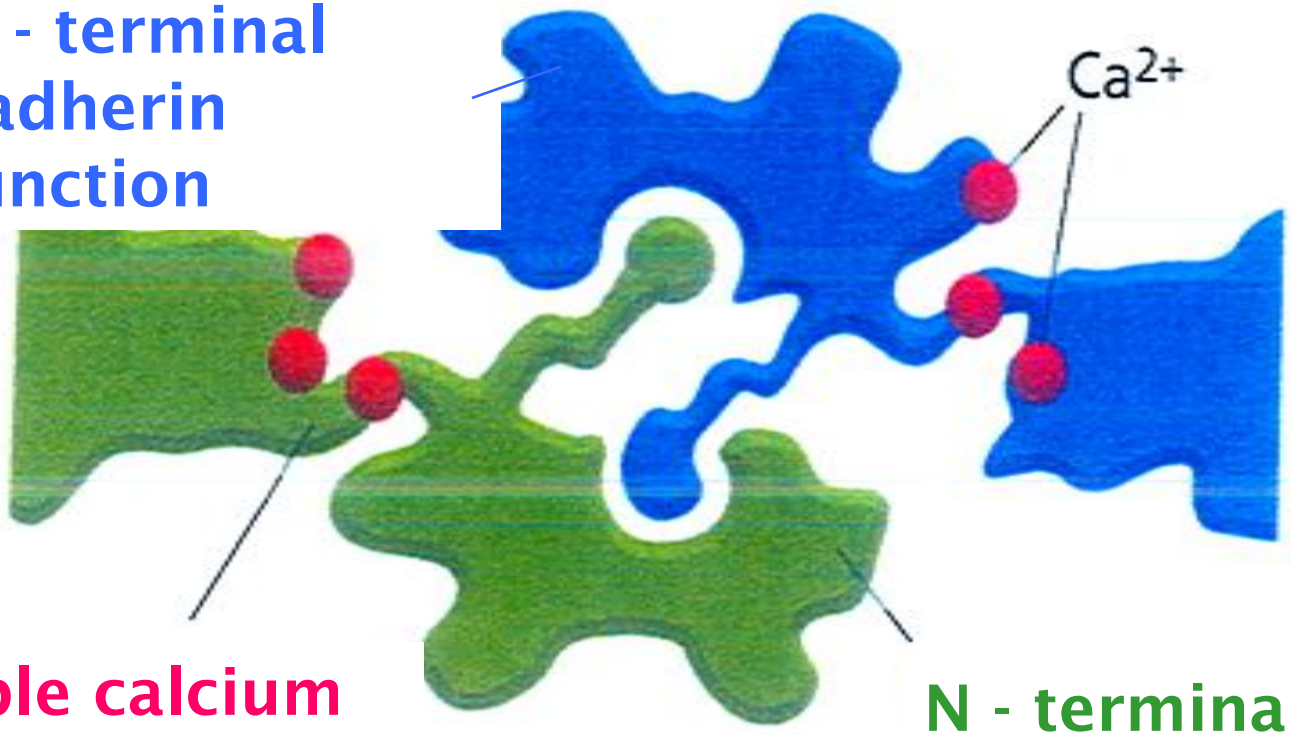
# Tight junctions binding cells




Classical adherin
(E-cadherin)
Fat-like cadherin
Seven-pass
transmembrane
(flamingo) cadherin
Protein kinase
cadherins
Desmosomal
cadherins
Cadherin 23
Protocadherins
T cadherins
Intracellular
Extracellular

# Coupling Between Cadherins from Two Neighbor Cells

N - terminal cadherin junction




Flexible calcium dependent hinges

N - terminal cadherin junction

# Gene-fold changes in a colon cancer cell line (SW480-ADH) after 48 hours exposure to 1,25 $(OH)_2$ vitamin $D_3$

Cytoskeleton/adhesion

| | |
|---|---|
| + 39 | Type II keratin (hHKb1) |
| + 14 | Gravin |
| + 12 | E-cadherin |
| + 7 | Keratin 15 |
| - 4 | Calgizzarin |

GTPases and related

| | |
|---|---|
| + 42 | RAB2 |
| + 21 | RA1BP1-interacting protein |
| + 4 | Breast cancer anti-estrogen resistance protein (BCAR3) |

Channels and transporters

| | |
|---|---|
| +30 | Putative monocarboxylate transporter (MCT) |
| +15 | 3-*beta*-hydroxysteroid dehydrogenase (3-*beta*-HSD) |

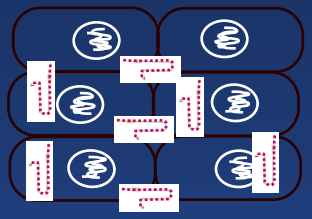
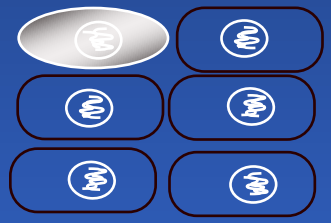
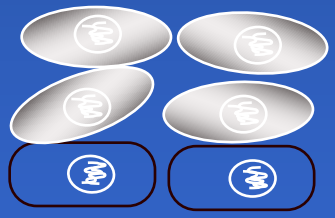
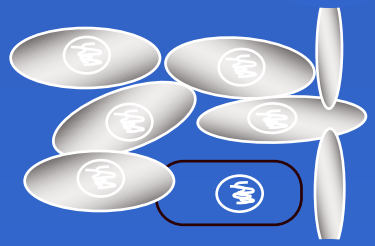
Apoptosis related

| | |
|---|---|
| +24 | Insulin-like growth factor binding protein-3 (IGFBP-3) |
| +11 | DAP-1 *alpha* |
| +10 | TNF-alpha converting enzyme |
| +7 | gadd45 |
| +6 | Ceramide glucosyltransferase |
| +6 | Prostate apoptosis response protein (par-4 ) |
| -5 | CD27BP (Siva) |
| +74 | 17-*beta*- hydroxysteroid dehydrogenase (17-HSD) |
| +20 | Cytochrome P450 III A |

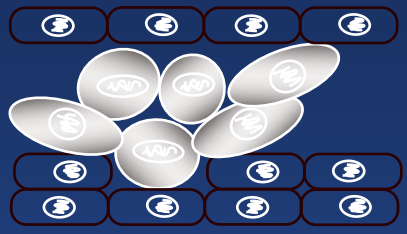
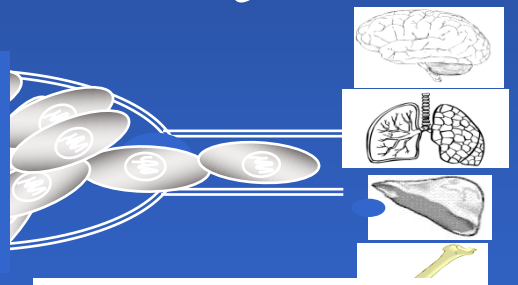
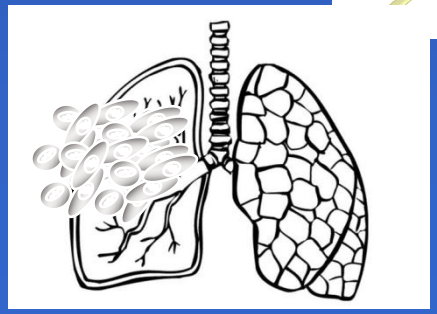
DNA cell cycle

| | |
|---|---|
| + 24 | $G_oS2$ |
| - 4 | Cyclin F |

# DINOMIT Theory of Cancer I

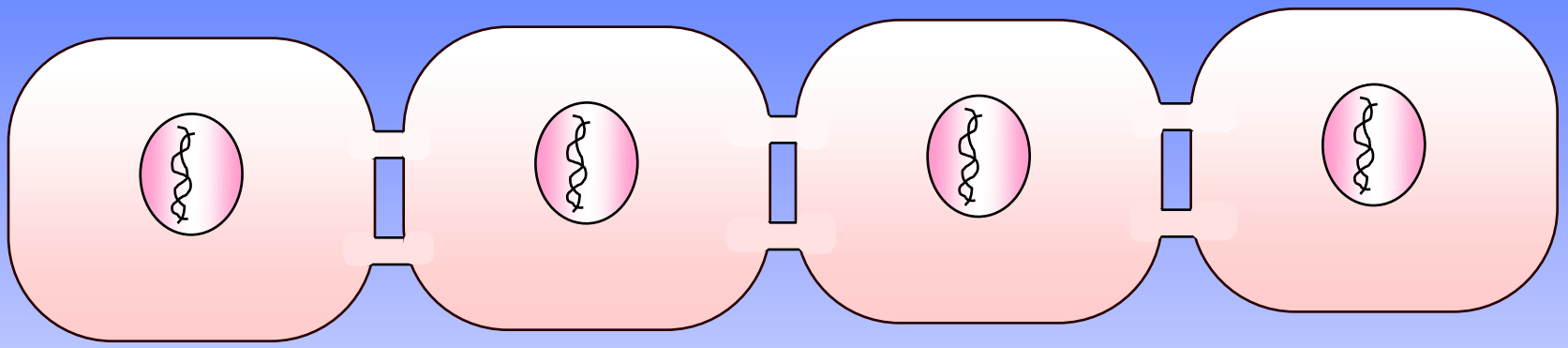
| Phase | Diagram | Process | Preventive or therapeutic Action |
|---|---|---|---|
| Vitamin D Replete (Normal) |  | Tight junctions intact Intercellular communication, growth inhibition and cell cycle normal non-mitotic | Maintain 25(OH) D level of 40-60 ng/ml |
| 1. Vitamin D Insufficiency Disjunction |  | Tight junctions weak or absent. Cells separate from each other very slightly. Cadherins lost or weak. Contact inhibition lost. Beta-catenins relocate. Natural selection begins. | Upregulation of tight junctions and cadherins by vitamin D metabolites |
| 2. Natural Selection |  | Natural selection favors reproduction of rapidly mitotic, aggressive cells. These appear as new stem cells (Wicha et al., 2008) | Vitamin D maintains tight junctions, contact inhibition, and normal growth and cell cycle |
| 3. Clonal Expansion |  | Rapidly mitotic, aggressive progeny predominate, a 1% advantage will fill compartment in 9000 generations | Vitamin D favors apoptosis and normal cell cycle |
| 4. Lysis and Penetration of Basement Membrane |  | Most aggressive cells compete for nutrients and oxygen, and penetrate basement membrane | Vitamin D inhibits lysis of basement membrane, Promotes sharing of micronutrients; Maintains intercellular junctions and desmosomes |

# DINOMIT Theory II

| Phase | Diagram | Description | Prevention or Therapeutic Action |
|---|---|---|---|
| 5. Stromal Phase |  | Invasion of Stroma | Re-establish tight junctions between cancer cells |
| 6. Lymphatic Entry Phase |  | Lymph vessel invasion | Re-establish tight junctions Prevent lymphatic entry |
| 7. Lymphatic Growth Phase |  | Lymph node colonization | Re-establish tight junctions Confine malignancy to lymph nodes |
| 8. Lymphatic Transport Phase |  | Lymphatic transport to brain, lung, liver, bone | None |
| 9. Metastasis (colonization) Phase |  | Malignant cells colonize remote host site | If VDR still present, re-establish tight junctions, downregulate VEGF, reduce growth rate, restore contact inhibition |

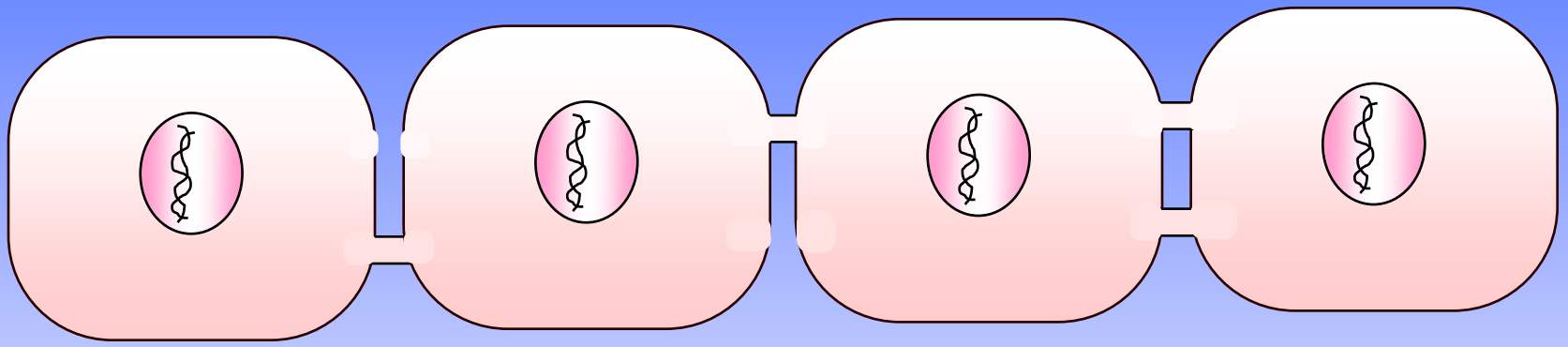

**You raised it from a mutant seed, you whack it!**

# DINOMIT



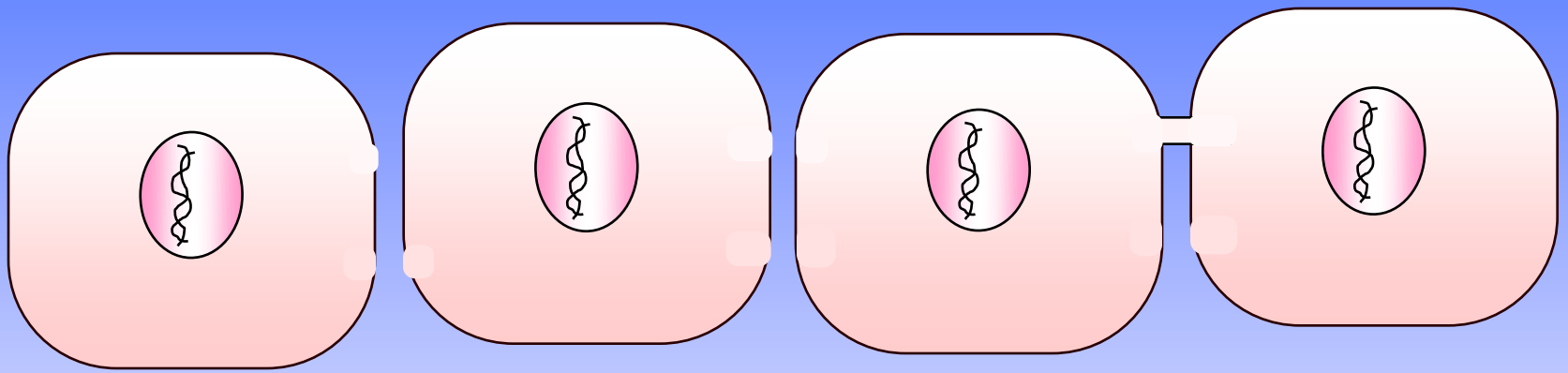
**Normally adherent cells**

# DINOMIT



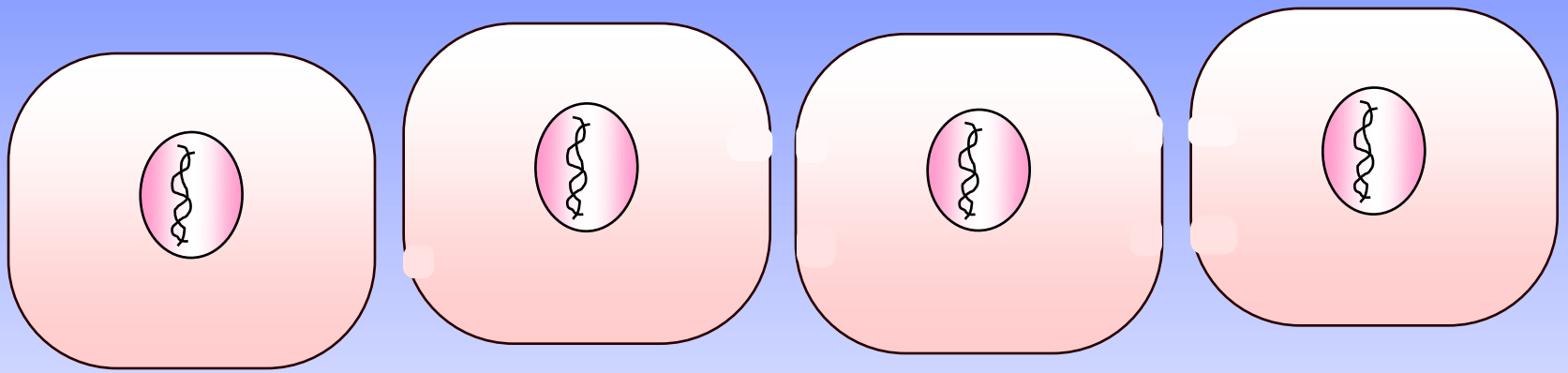
**Decoupling:  Loss of tight junctions**

# DINOMIT



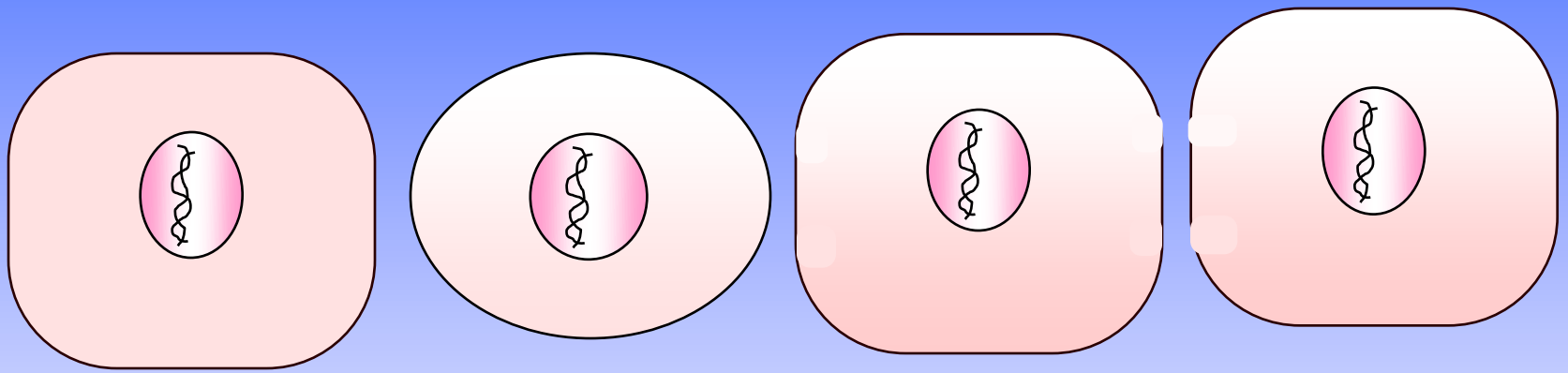
**Decoupling advances**

# DINOMIT



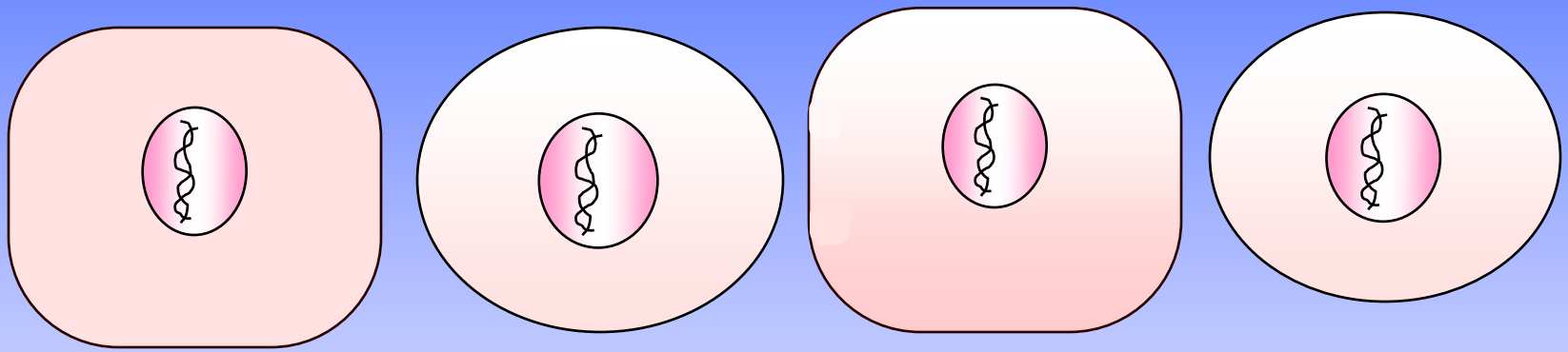
**Decoupling becomes complete**

# *D*INOMIT- Disjunction



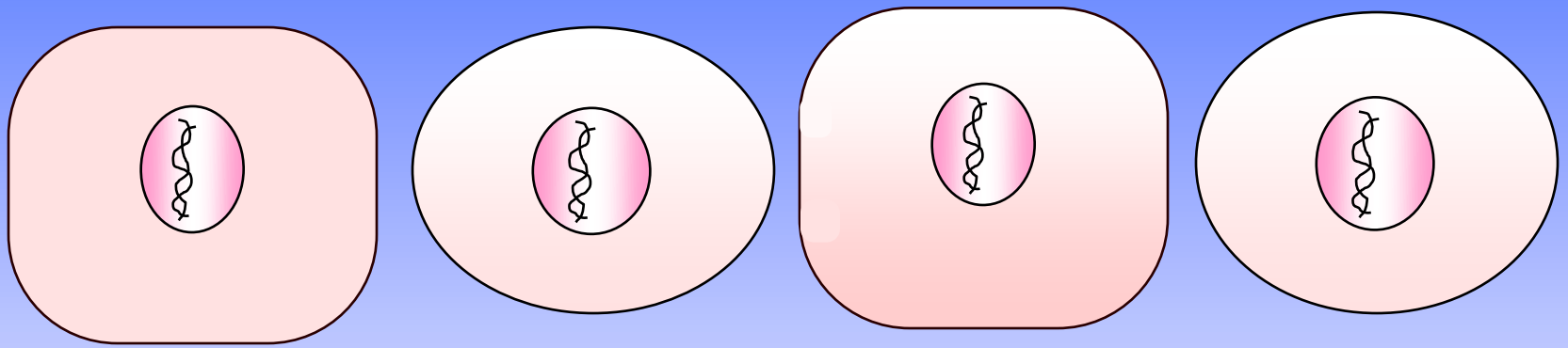
**Mild Dysplasia due to loss of tight junctions**

# D/NOMIT-Initiation



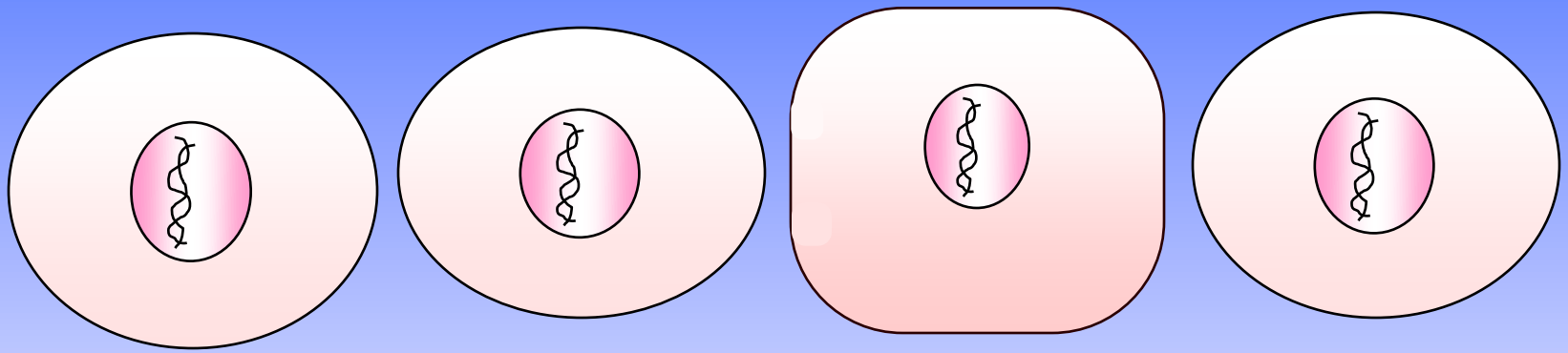
**DNA variation due to infidelity of reproduction or carcinogens**

# D/NOMIT-Initiation



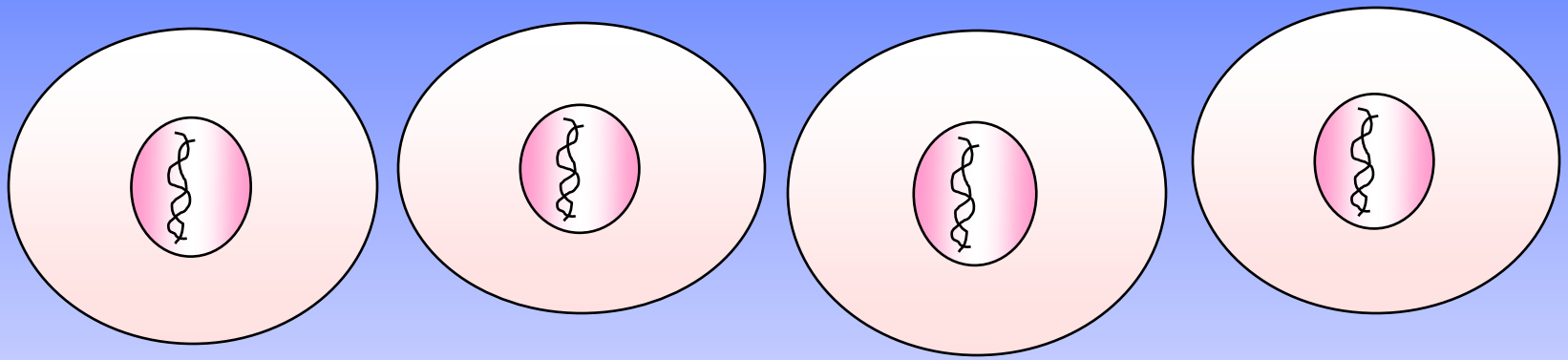
**Continued variation in DNA and epigenetics**

# D*I*NOMIT-Initiation



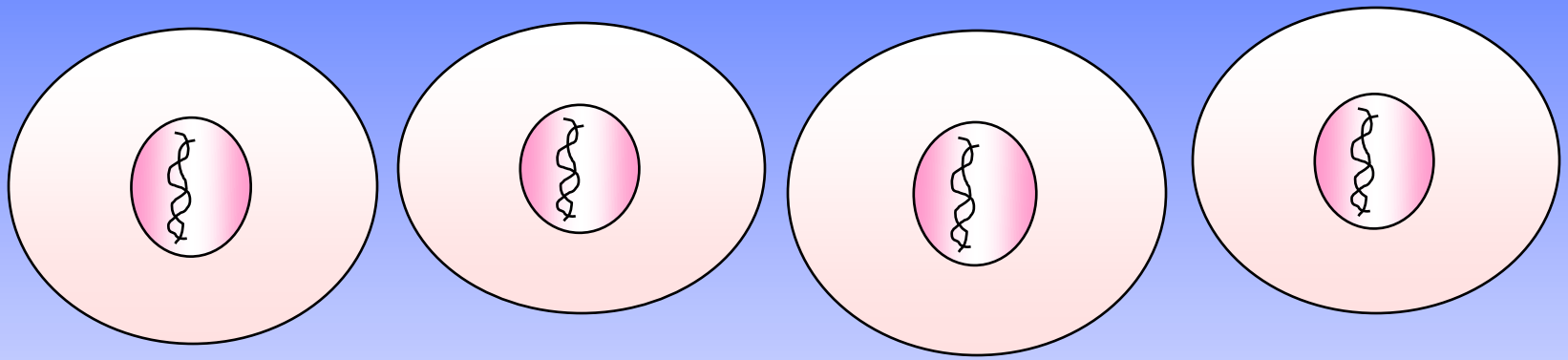
**Continued variation in DNA and epigenetics**

# DI*N*OMIT-Natural Selection



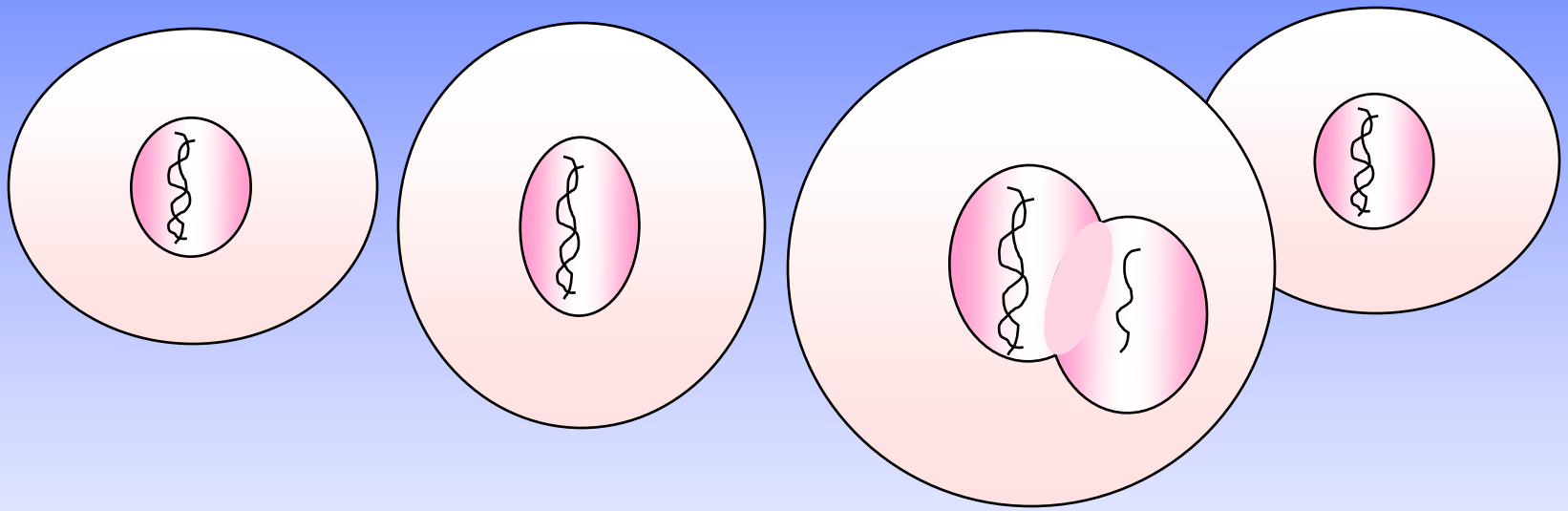
**Natural selection >> rapidly reproducing clones**

# DI*N*OMIT-Natural Selection



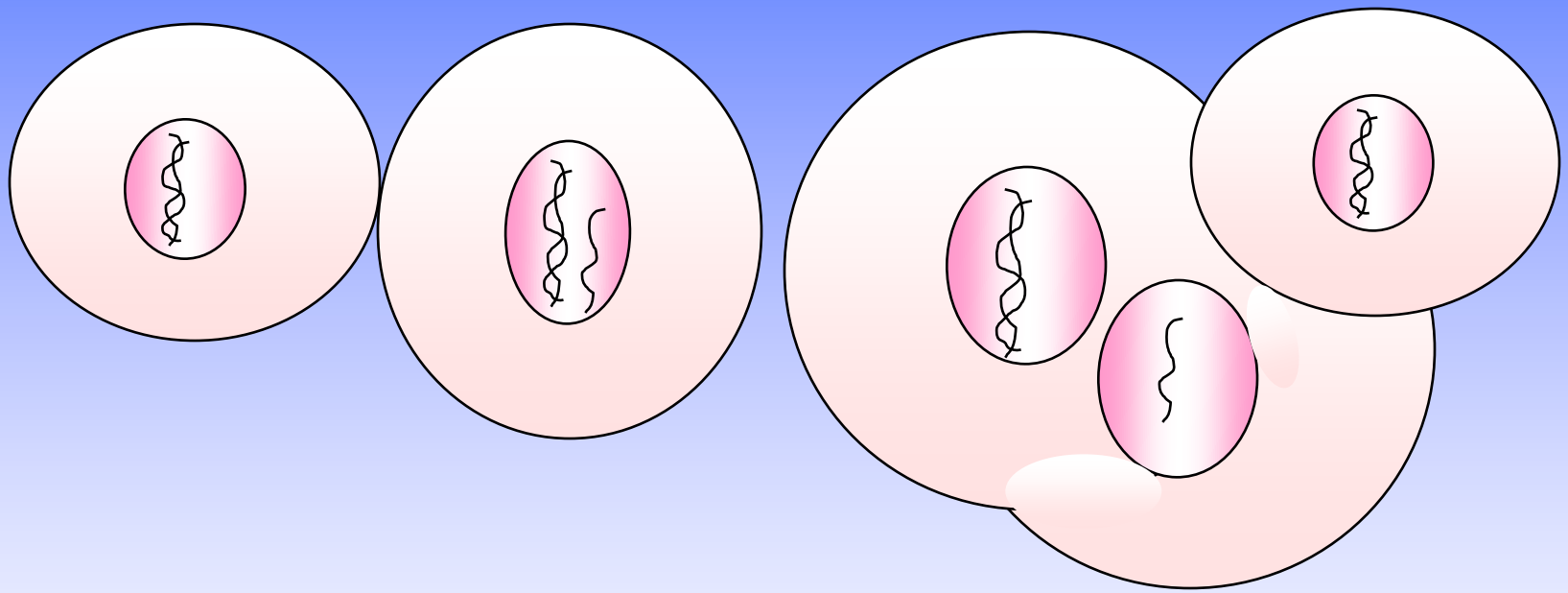
**Natural selection >> rapidly reproducing clones**

# DIN*O*MIT-Overgrowth



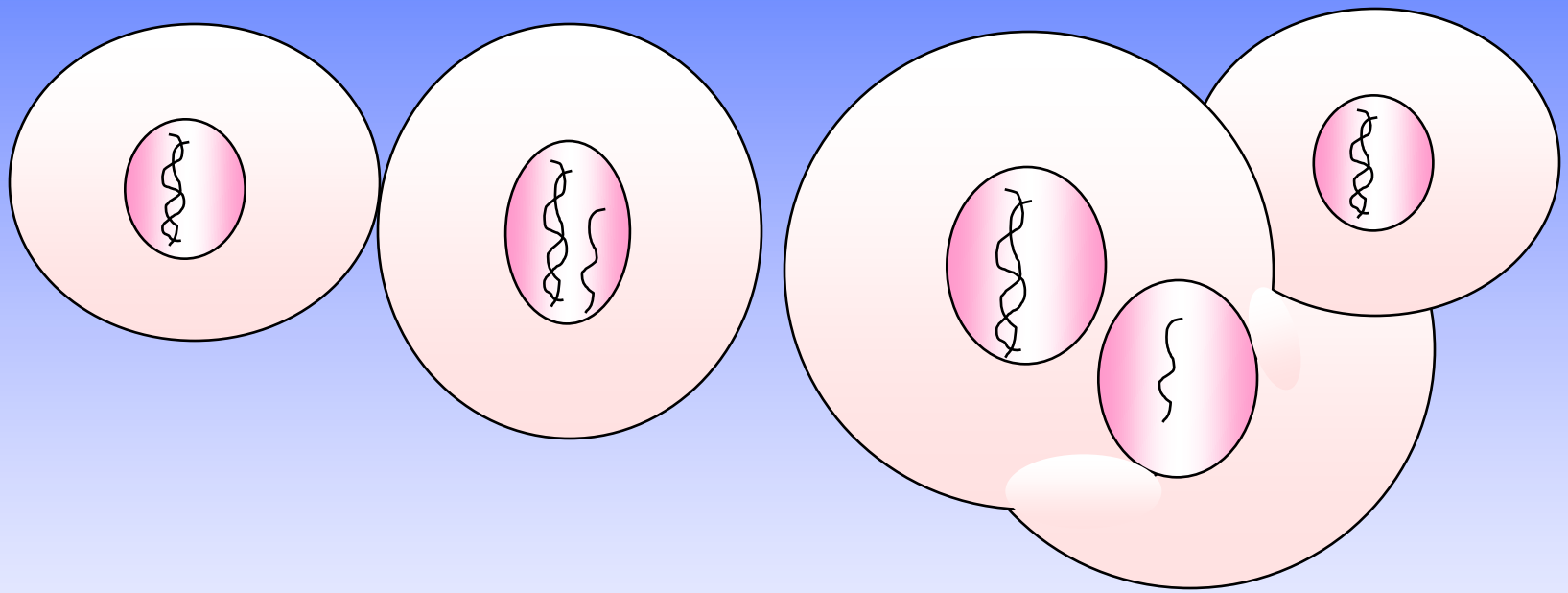
**New clone rapidly mitotic**

# DIN*O*MIT-Overgrowth



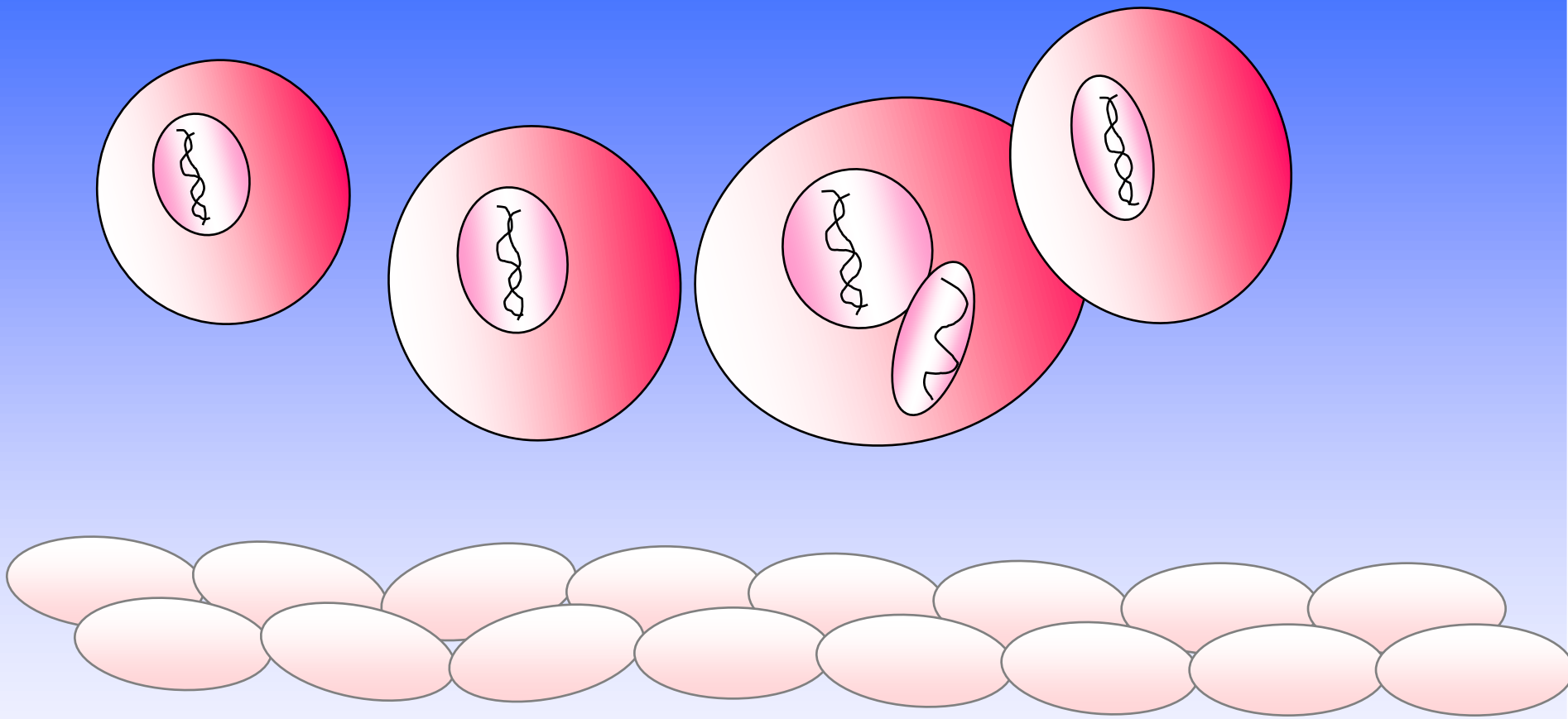
**Infidelity of DNA and epigenetics**

# DIN*O*MIT-Overgrowth



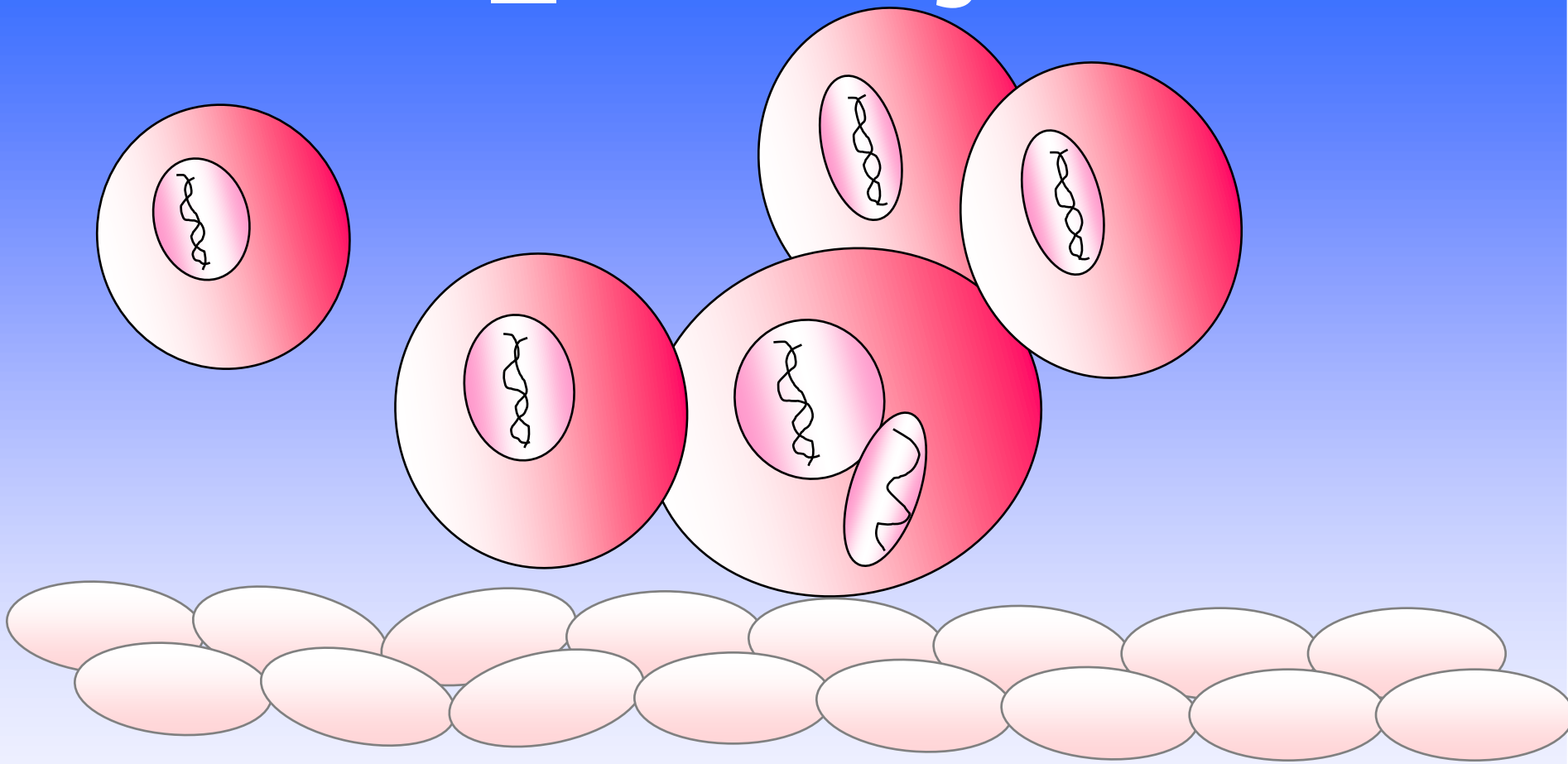
**Infidelity of DNA and epigenetics**

# DIN*O*MIT-Overgrowth



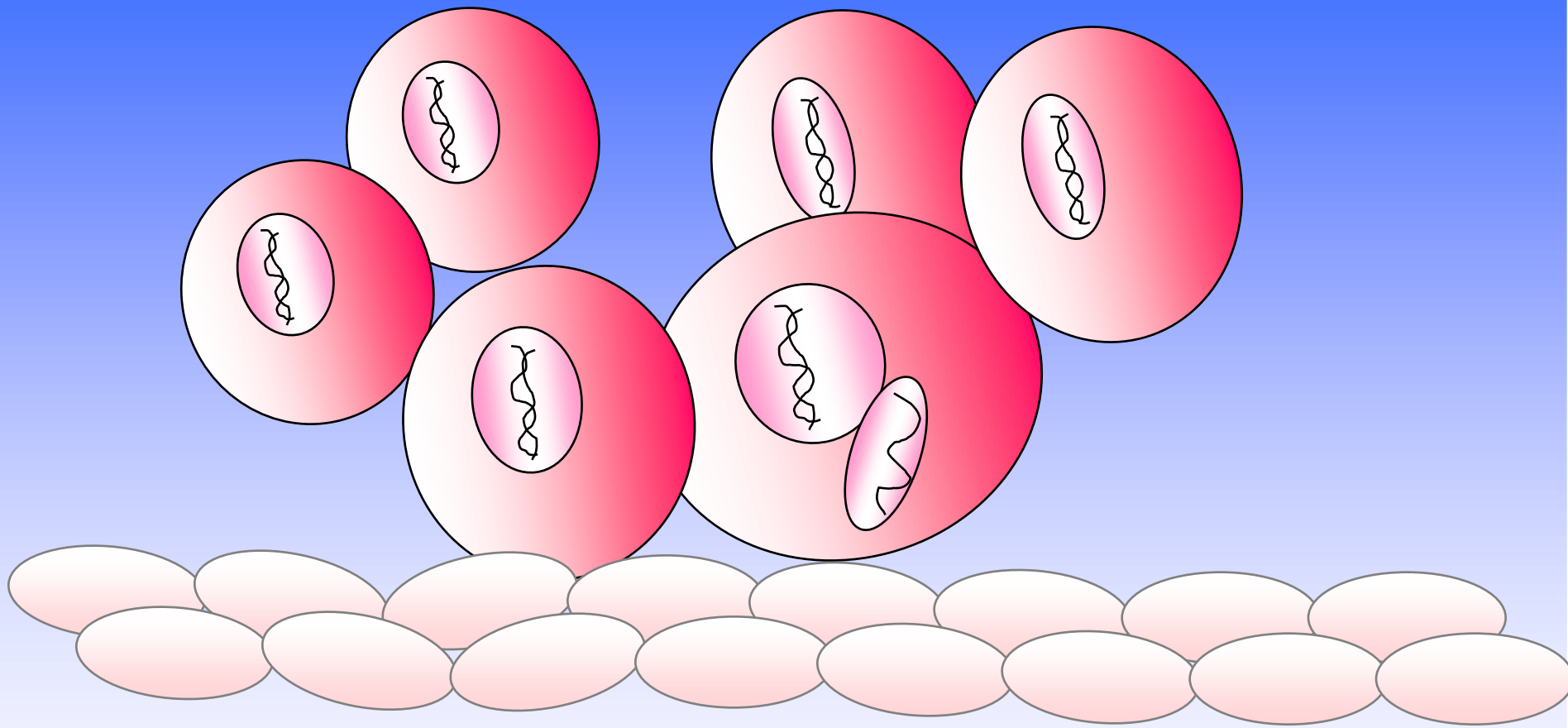
**Overgrowth creates crowding**

# DIN*O*MIT-Overgrowth



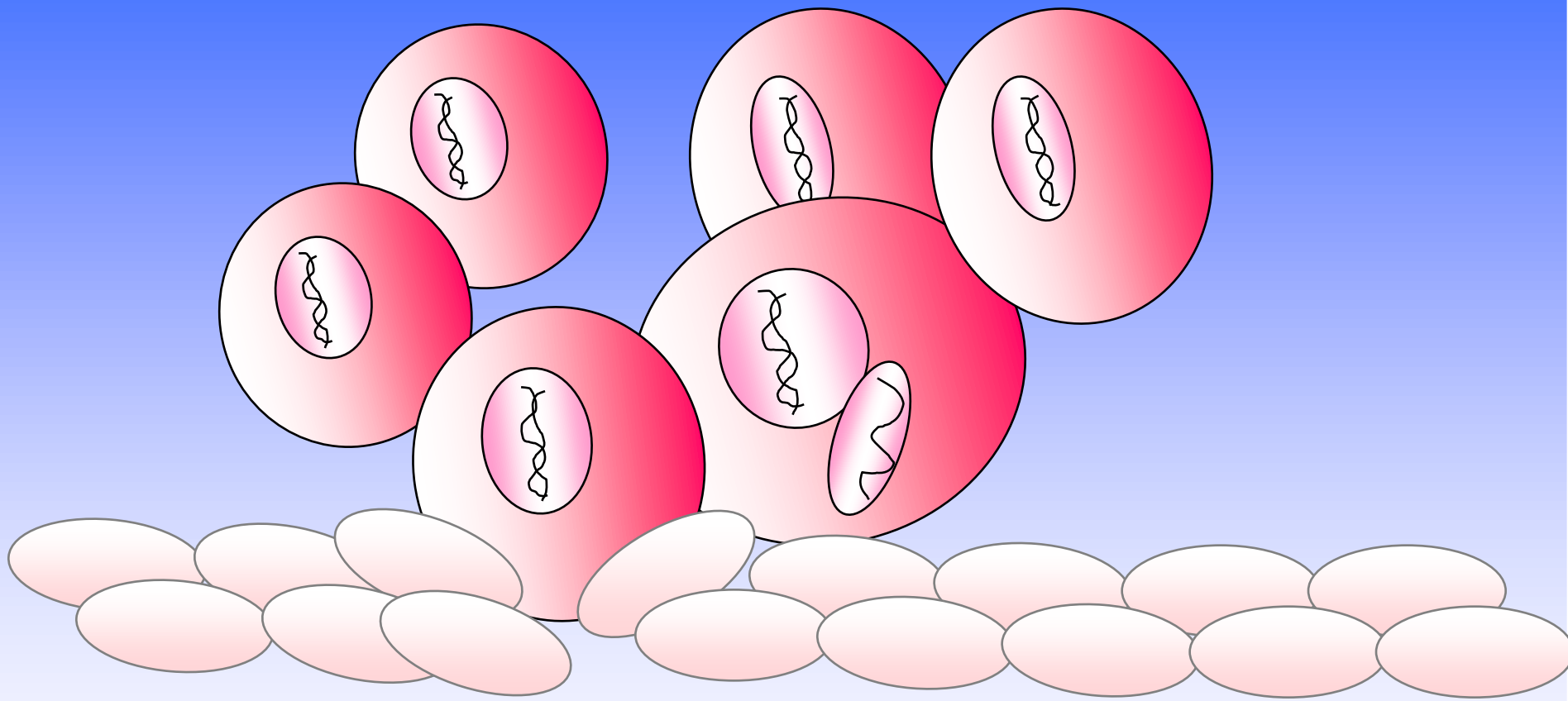
**Overgrowth creates crowding**

# DIN*O*MIT-Overgrowth



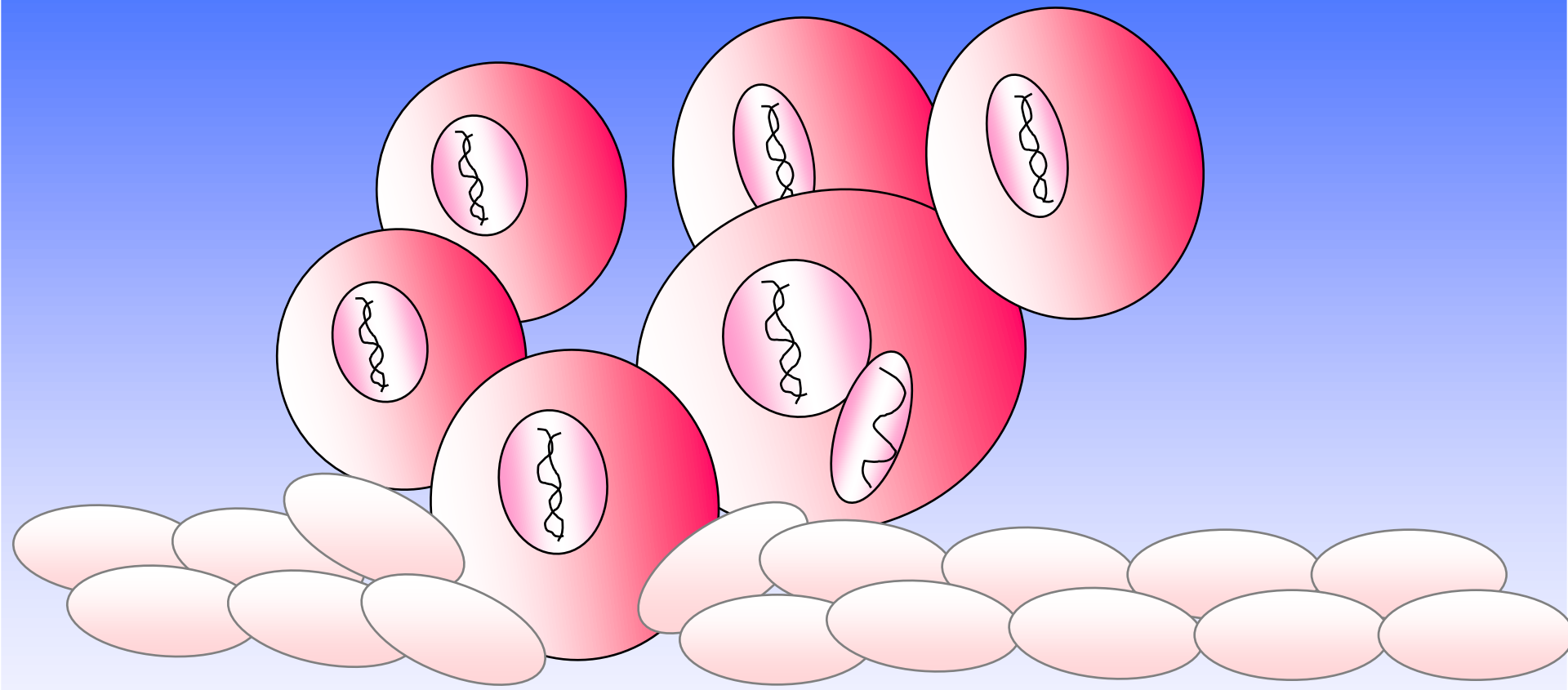
**Beginning penetration of basement membrane**

# DIN*O*MIT-Overgrowth



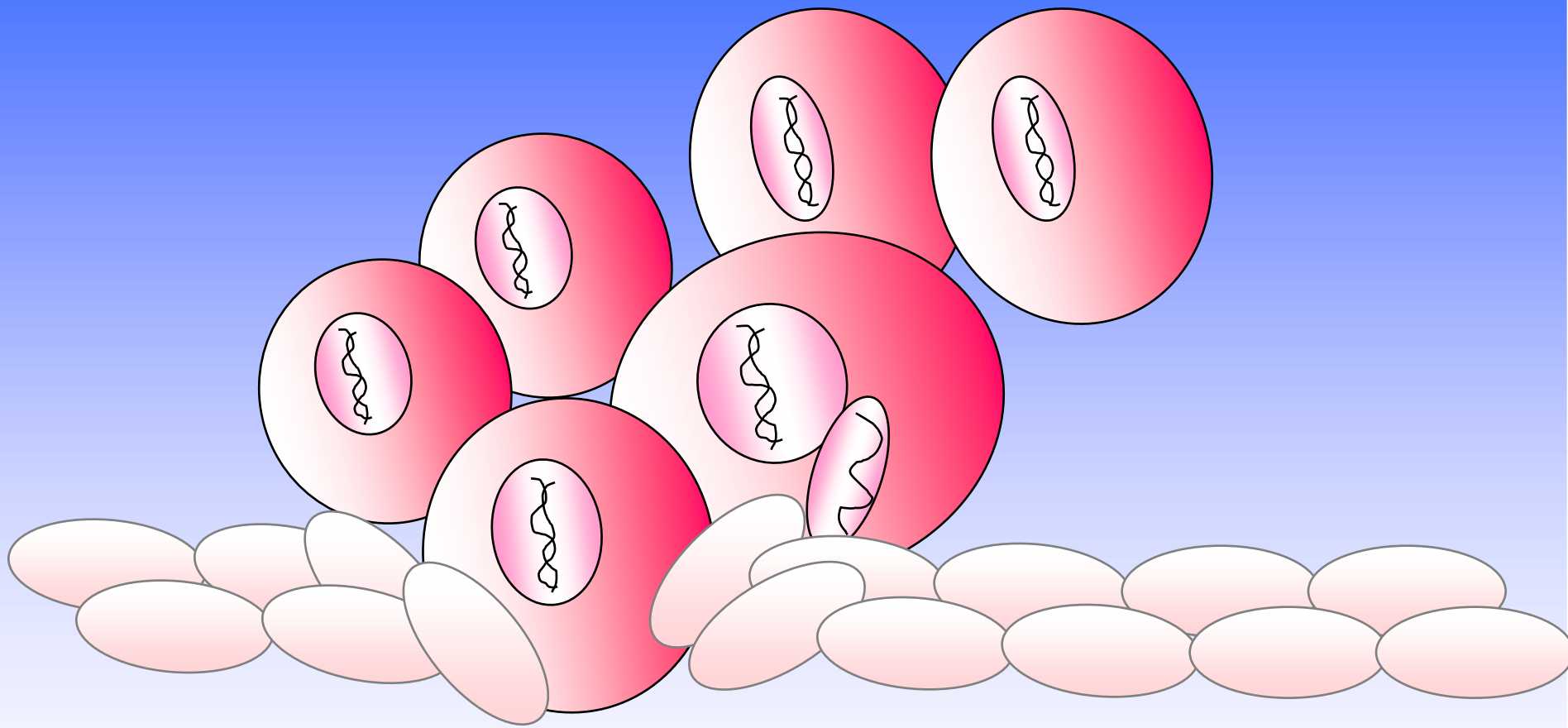
**Ongoing penetration of basement membrane**

# DIN*O*MIT-Overgrowth



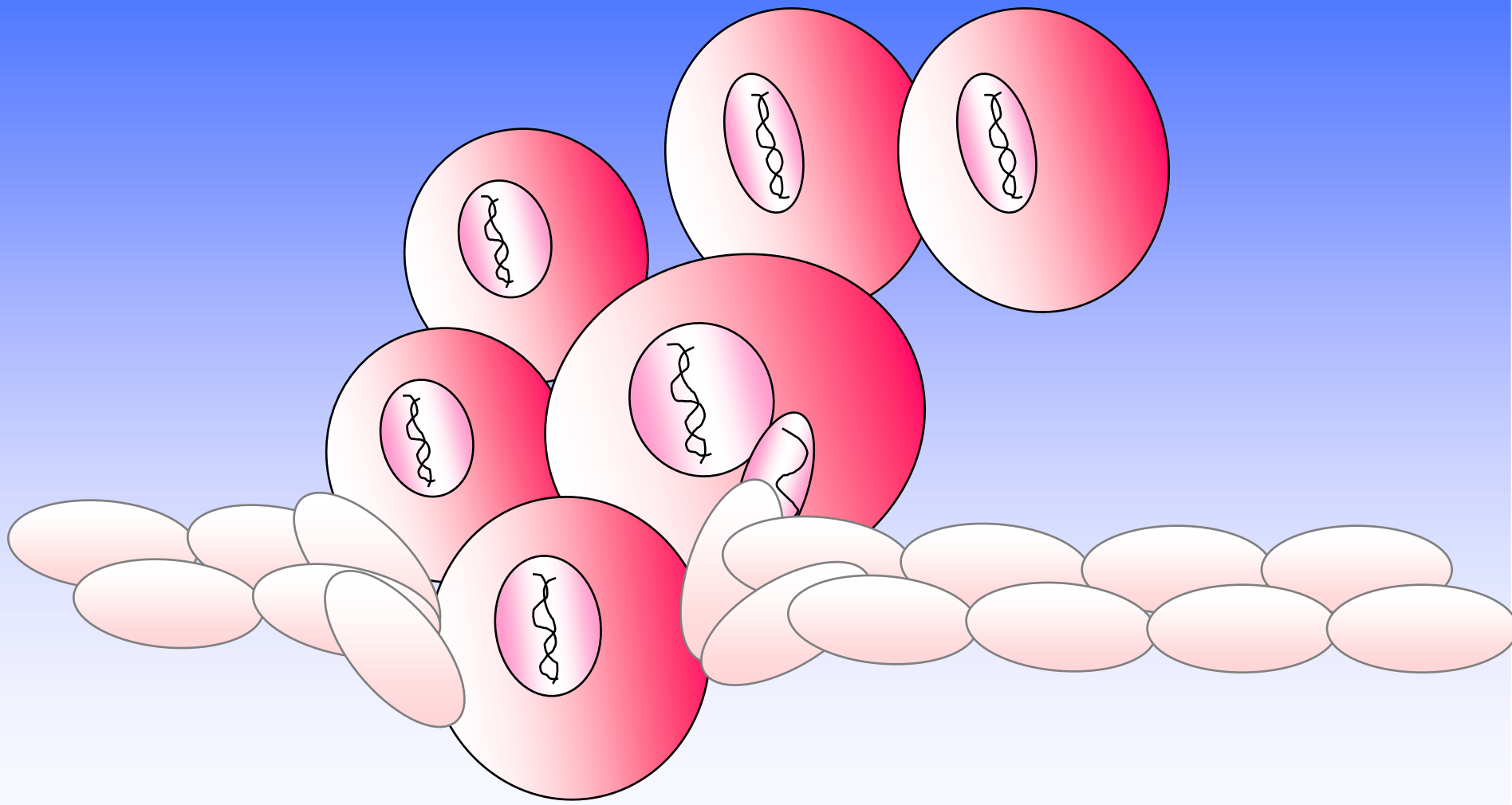
**Fuller penetration of basement membrane**

# DIN*O*MIT-Overgrowth



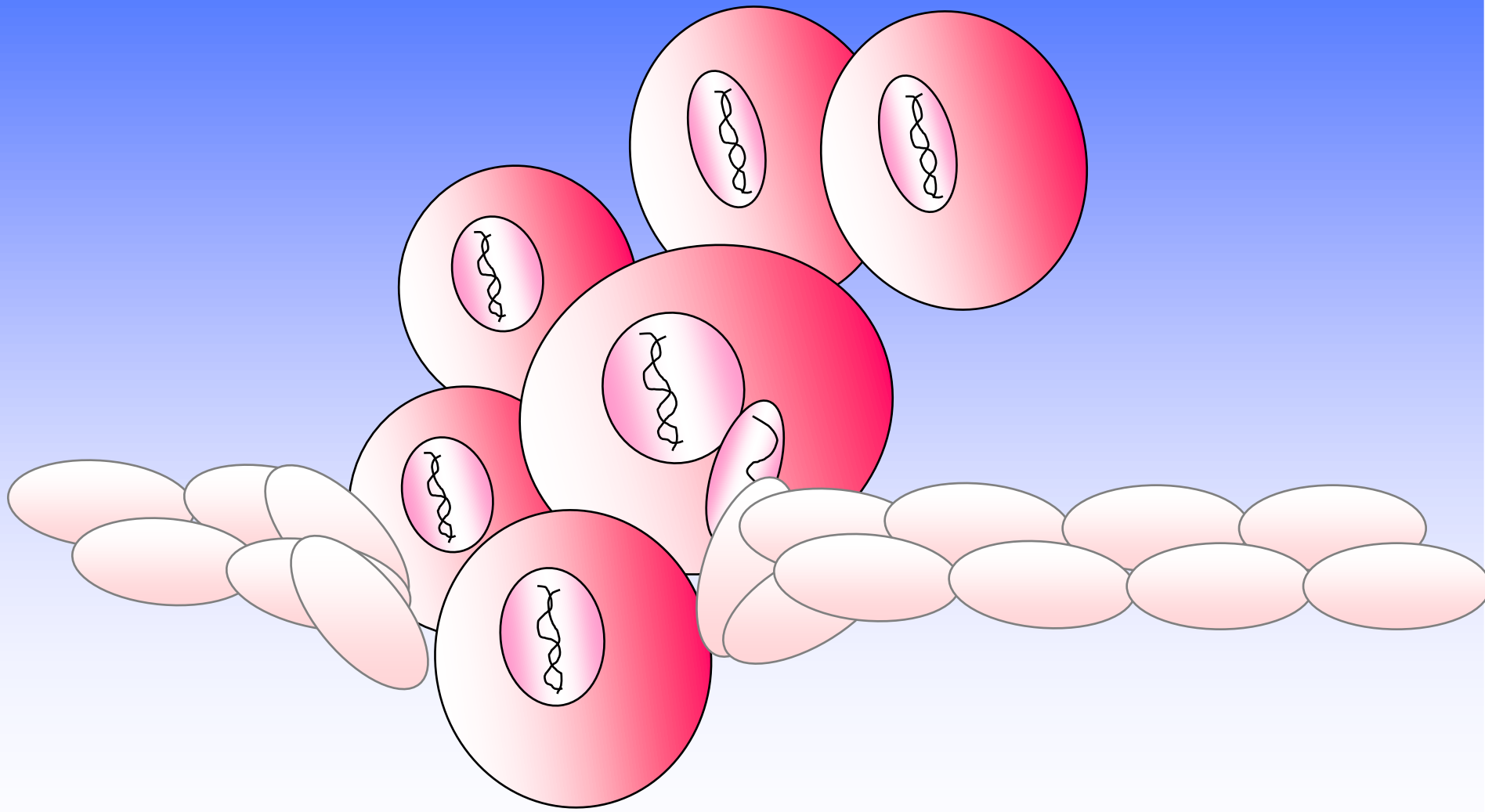
**Penetration of basement membrane continues**

# DIN*O*MIT-Overgrowth



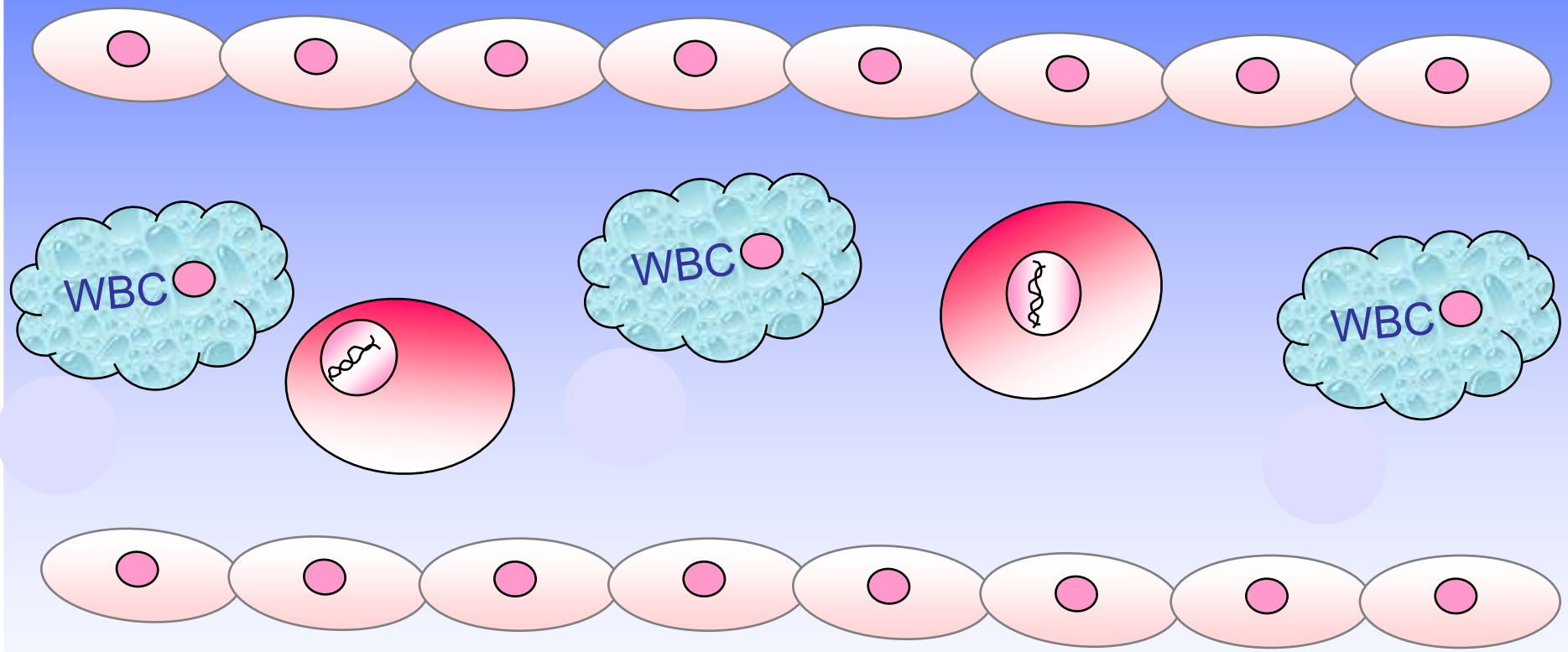
**Penetration of basement membrane continues**

# DINOMIT-Overgrowth



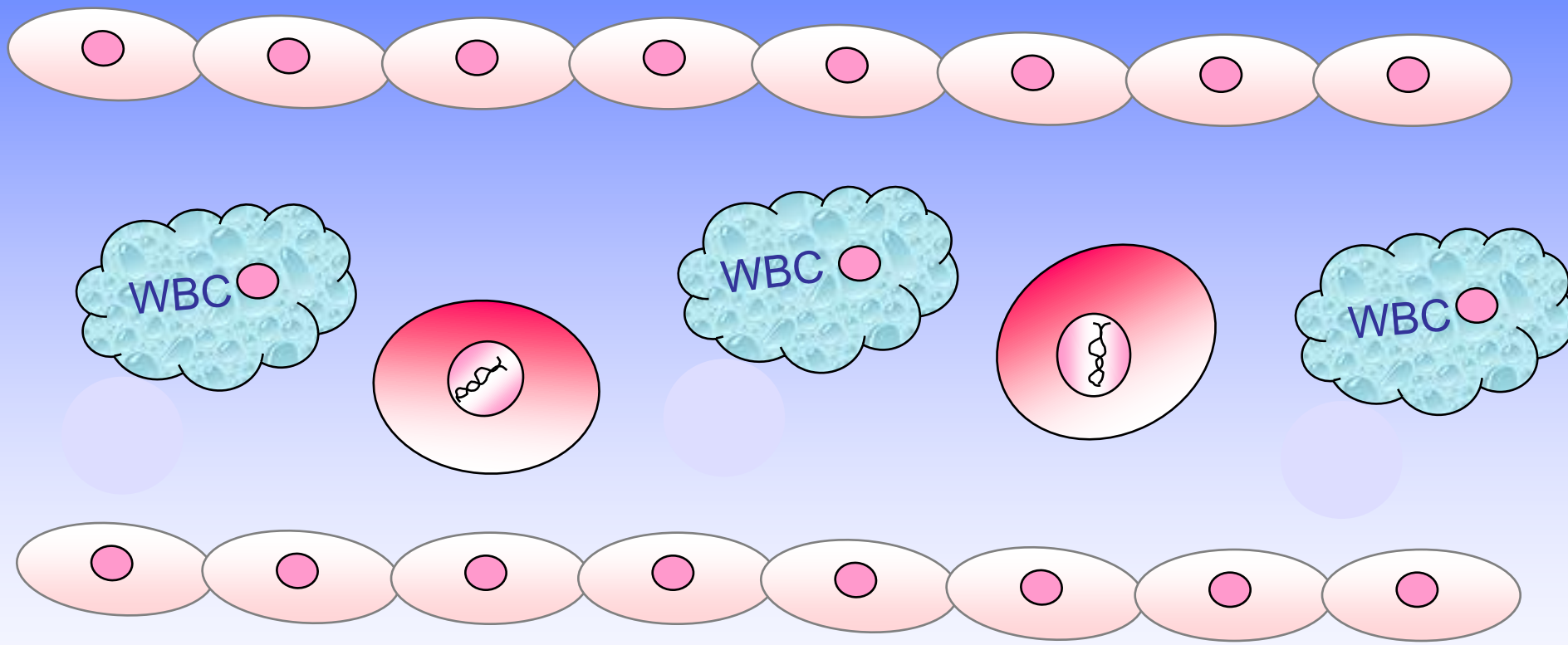
**Penetration of basement membrane continues**

# DINO*M*IT-Metastasis



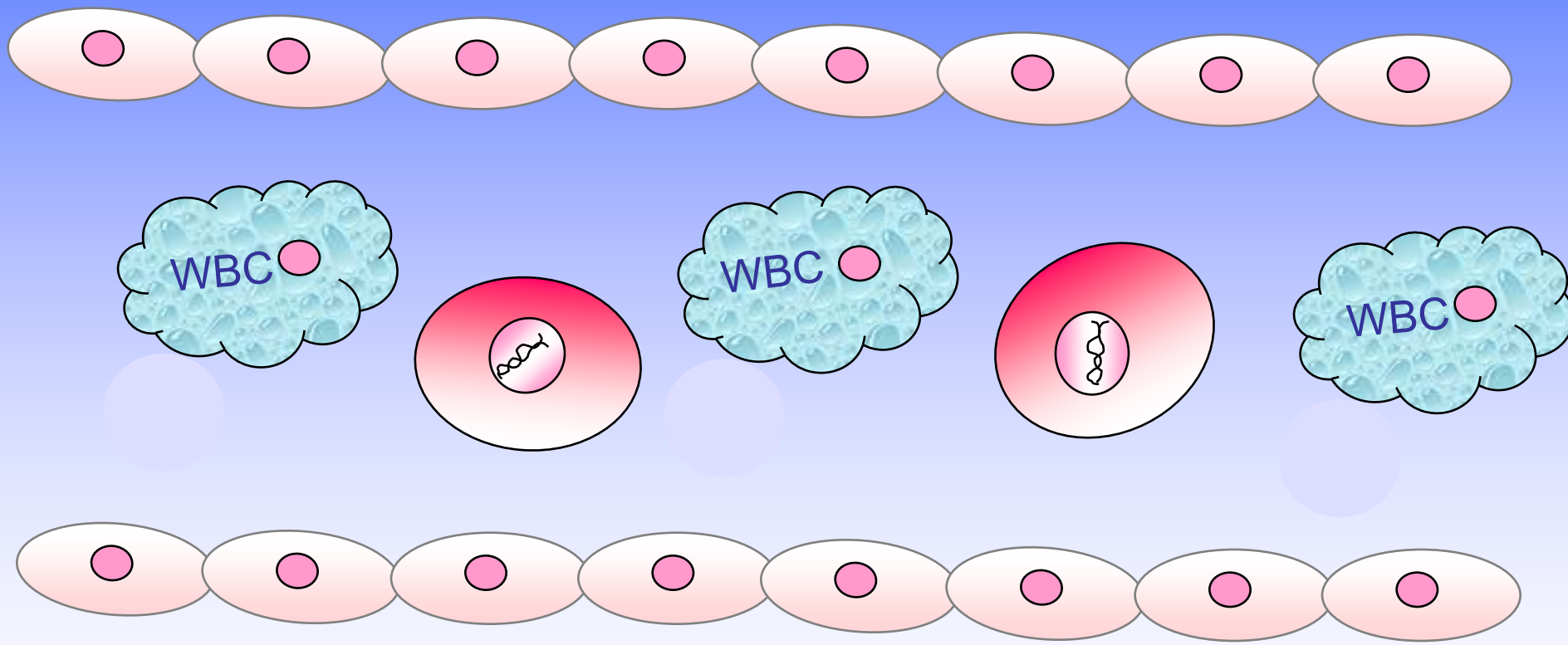

**Malignant cells enter lymphatic circulation**

# DINO*M*IT-Metastasis



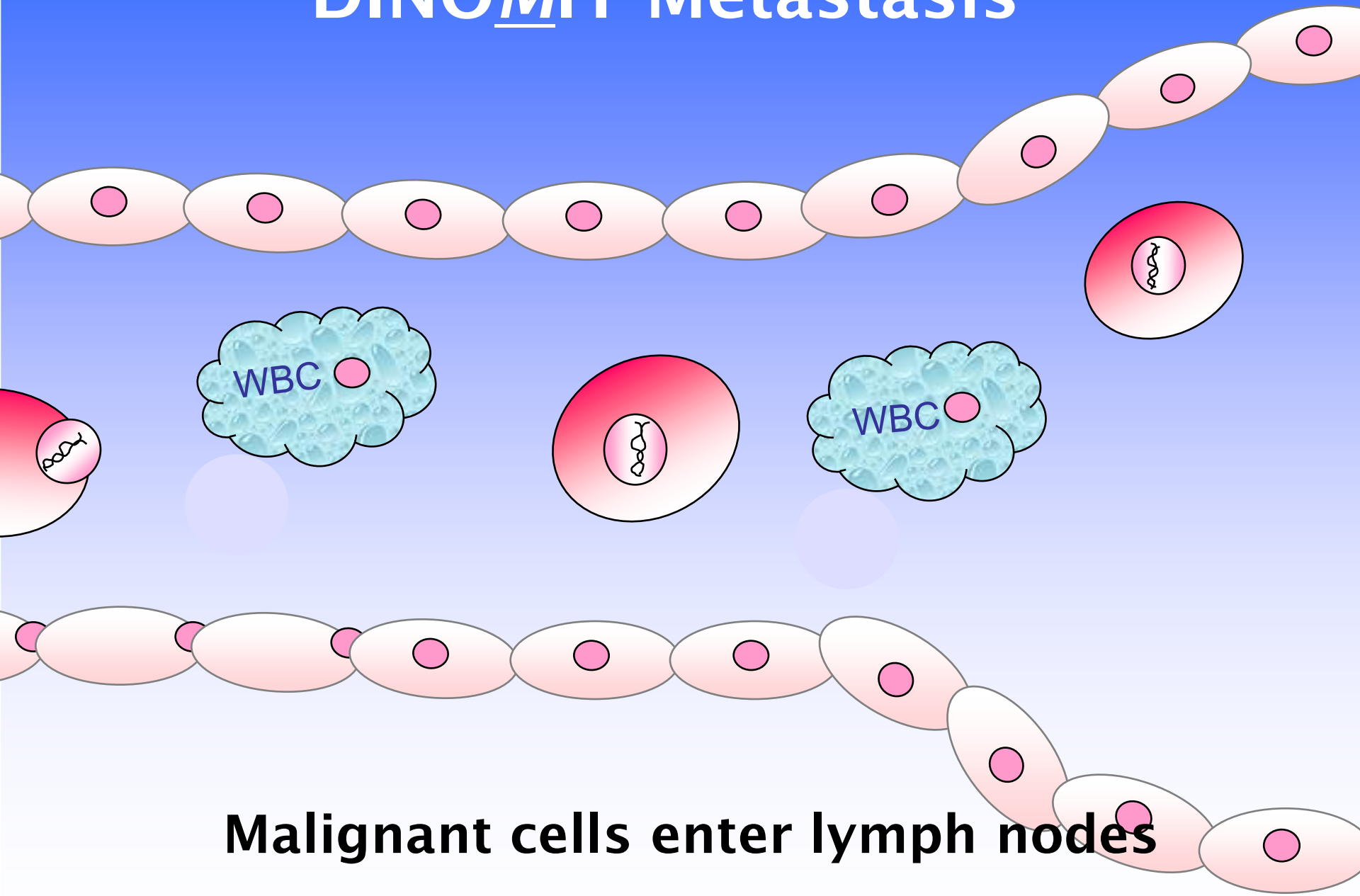

**Malignant cells enter lymphatic circulation**

# DINO*M*IT-Metastasis



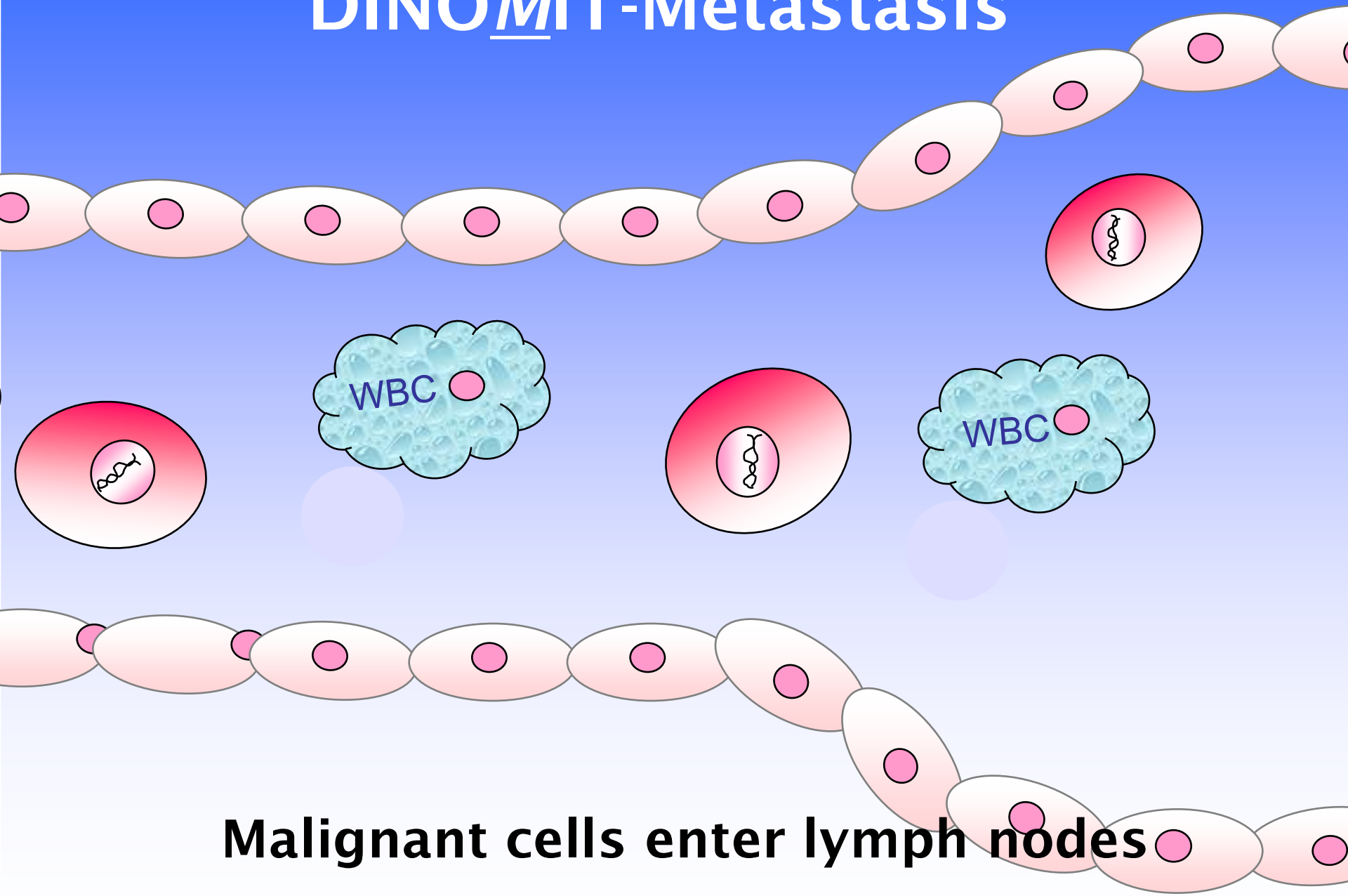

**Malignant cells enter lymphatic circulation**

# DINO*M*IT-Metastasis



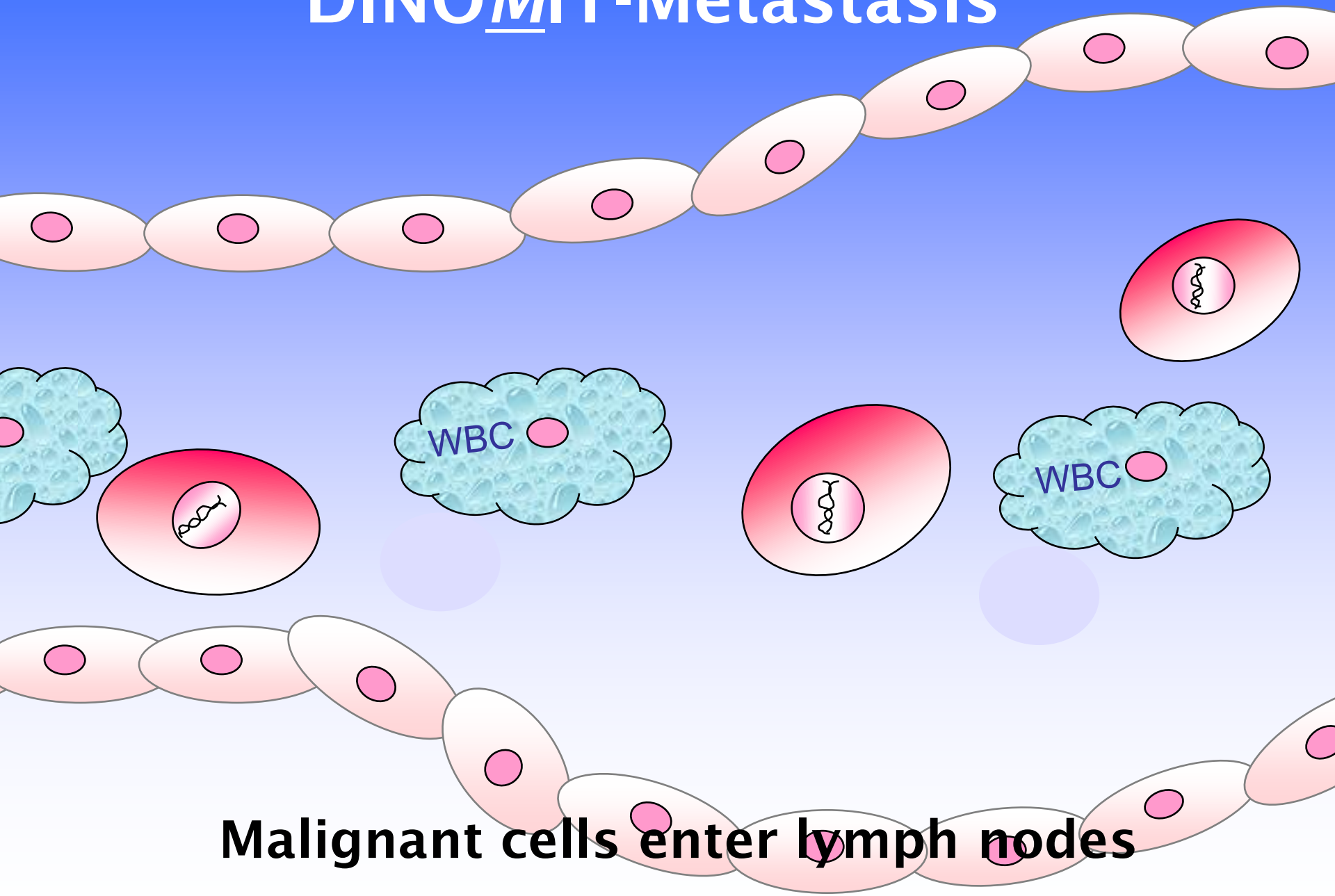

**Malignant cells enter lymph nodes**

# DINO*M*IT-Metastasis



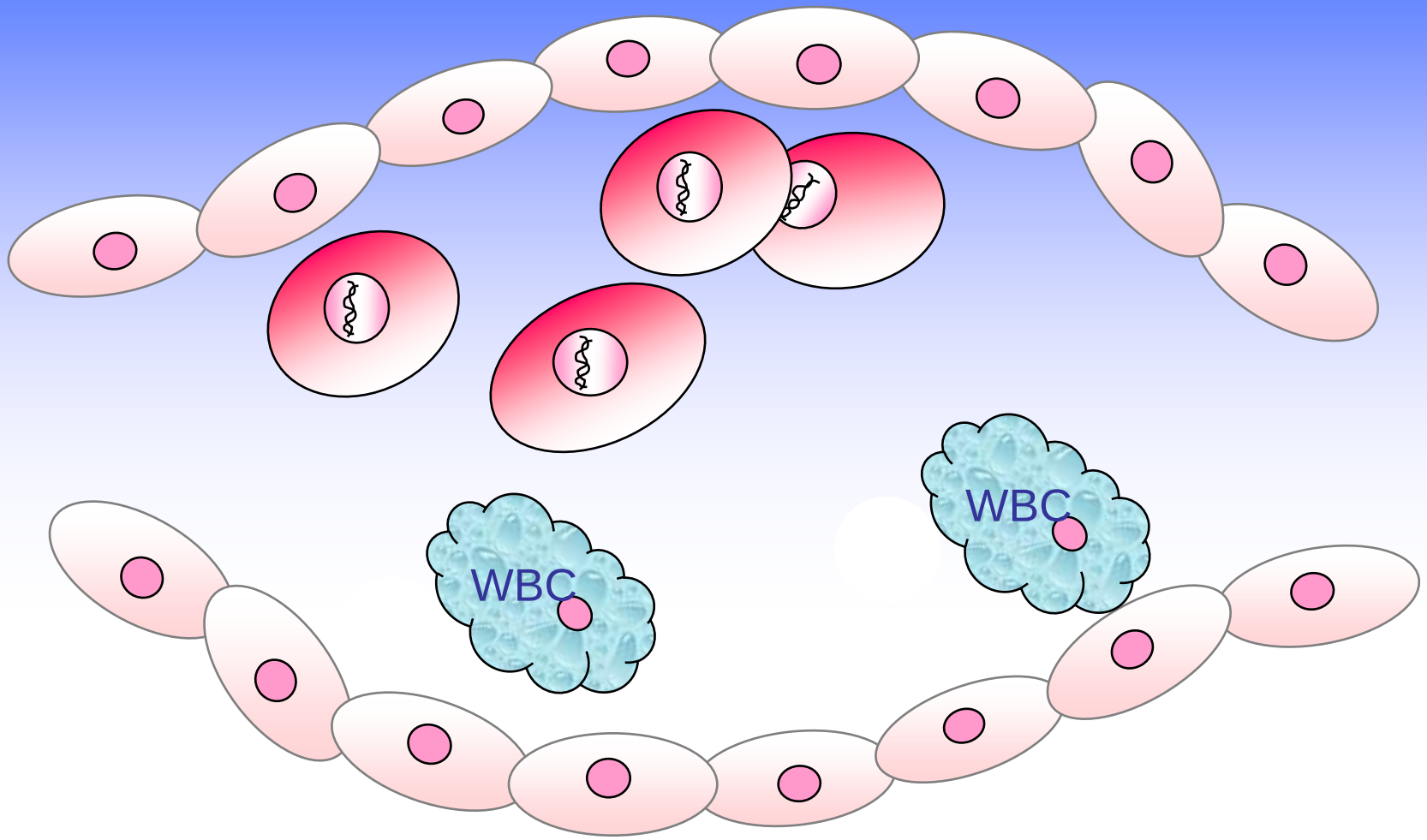

**Malignant cells enter lymph nodes**

# DINO*M*IT-Metastasis



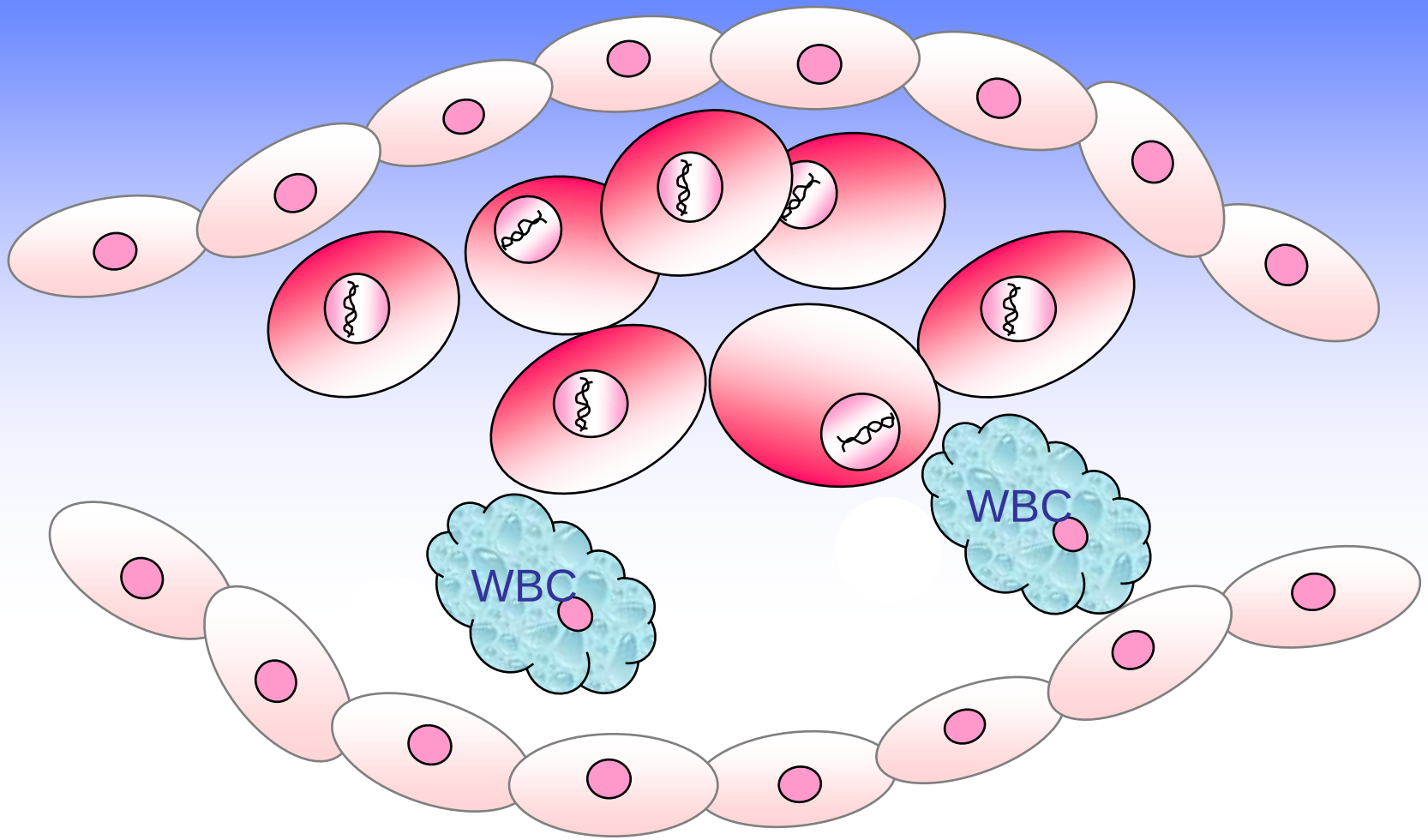

**Malignant cells enter lymph nodes**

# DINO*M*IT-Metastasis



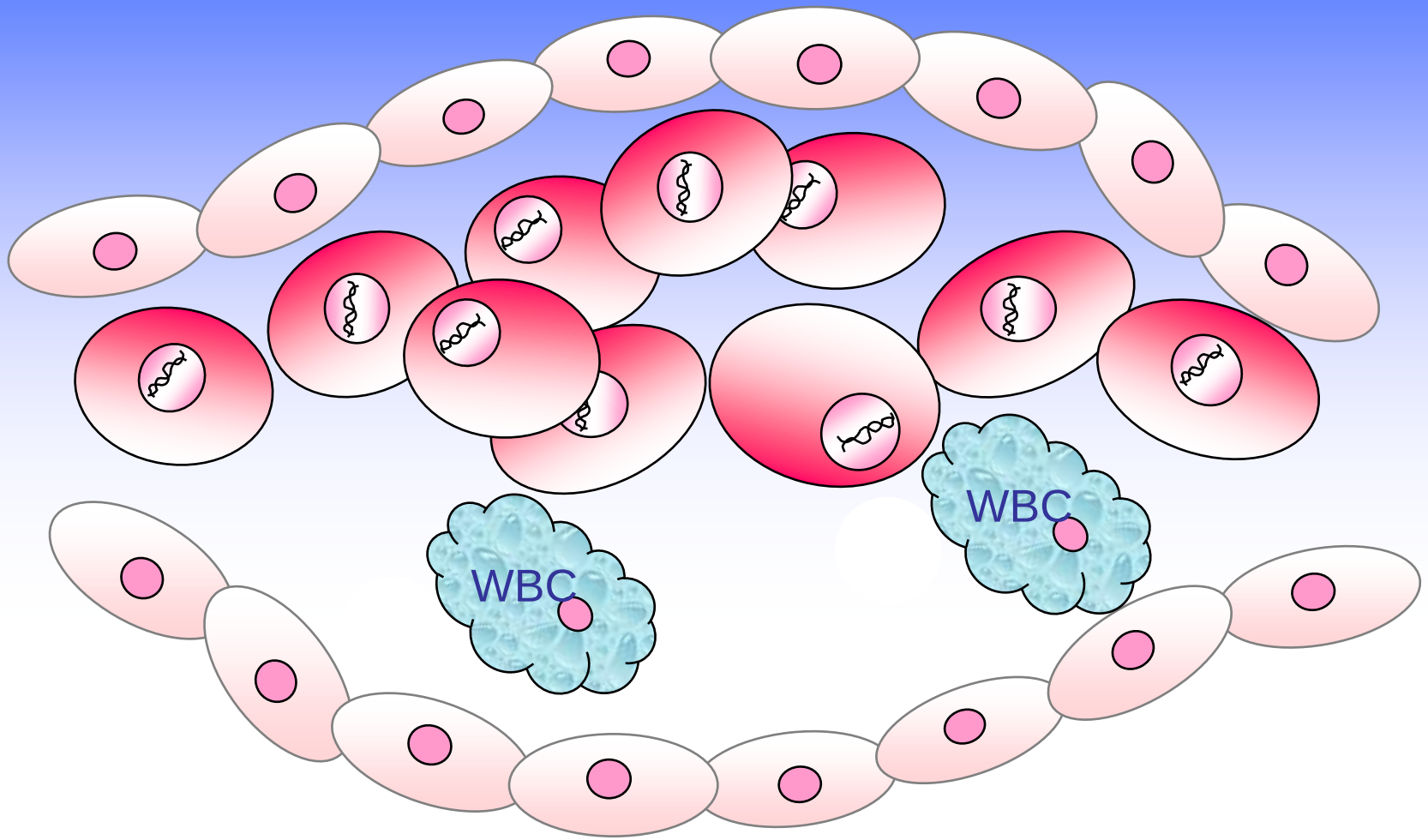

**Malignant cell population grows**

# DINO*M*IT-Metastasis



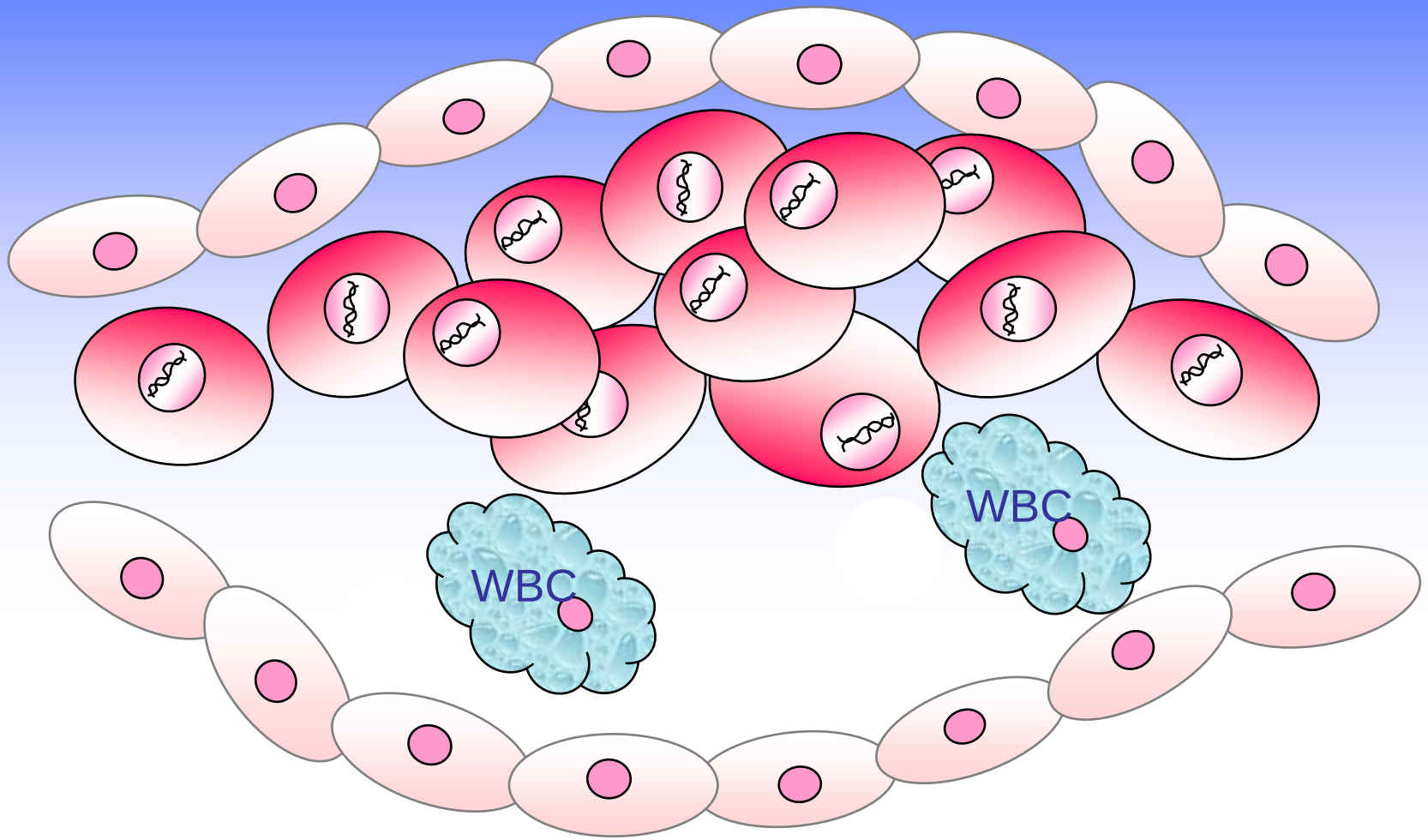

**Expansion of malignant clone in lymph node**

# DINO*M*IT-Metastasis



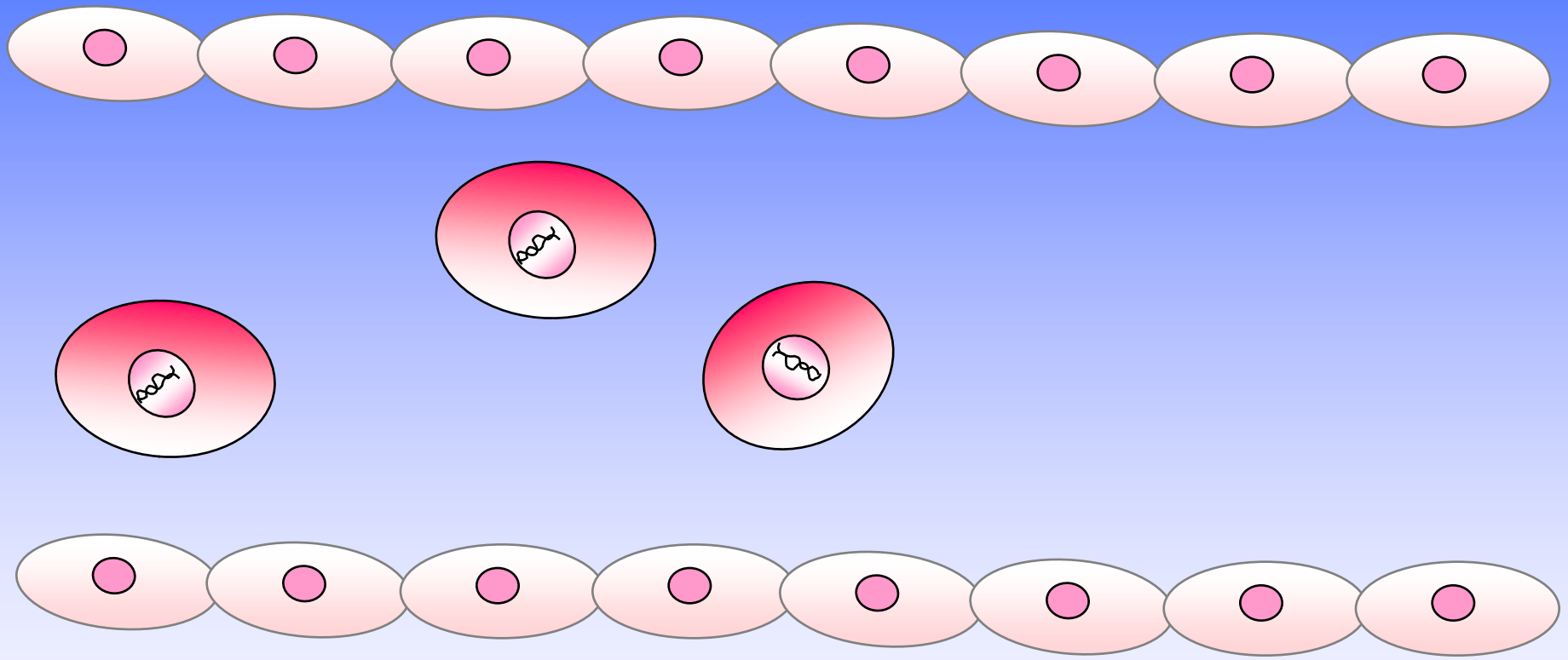

**Expansion of malignant clone in lymph node**

# DINO*M*IT-Metastasis



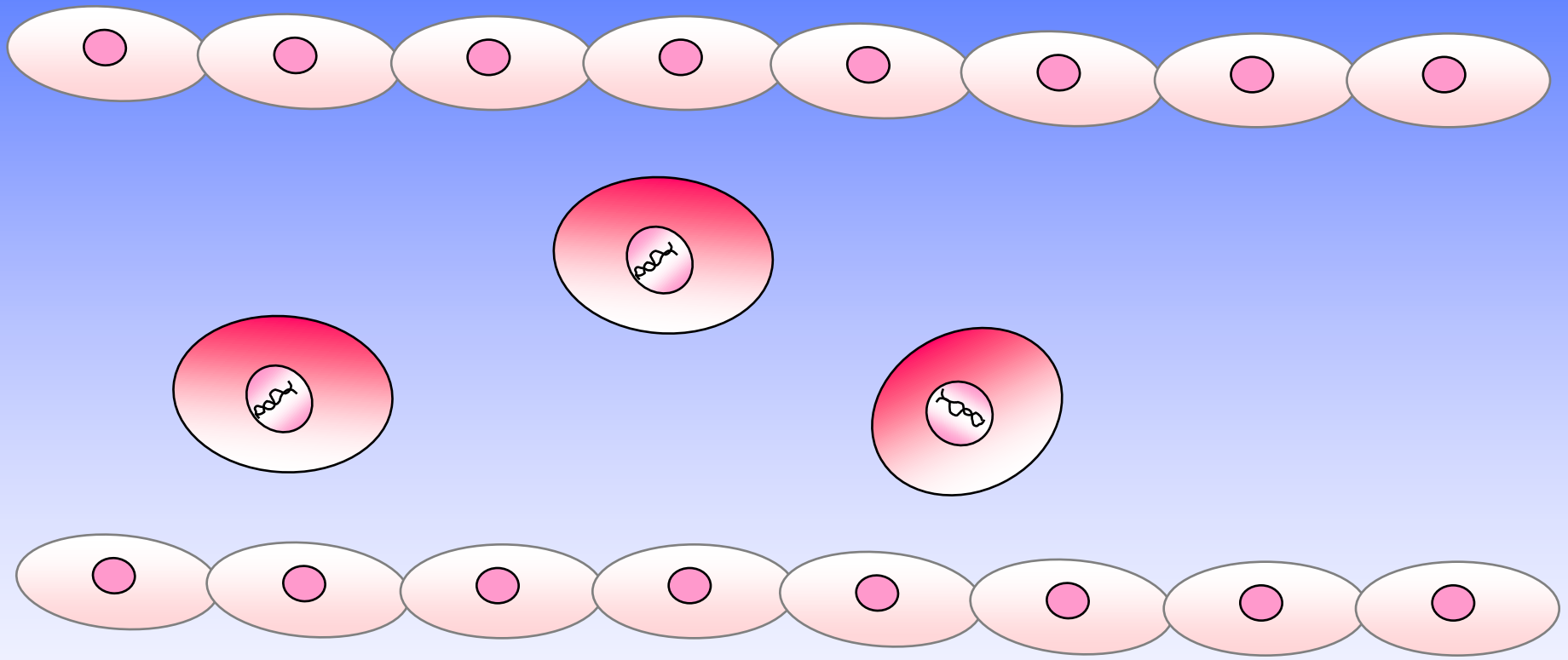

**Expansion of malignant clone in lymph node**

# DINO*M*IT-Metastasis



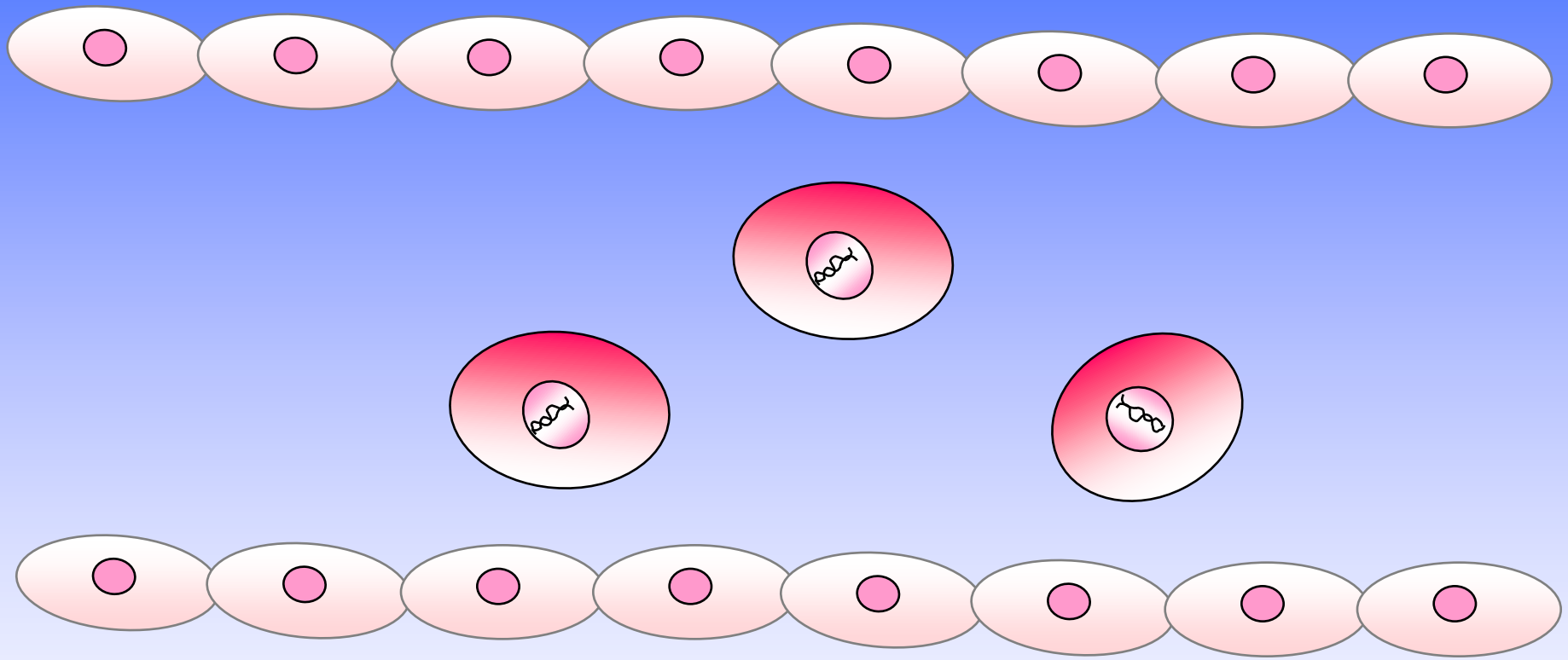
**Malignant cells transported from lymph node**

# DINO*M*IT-Metastasis



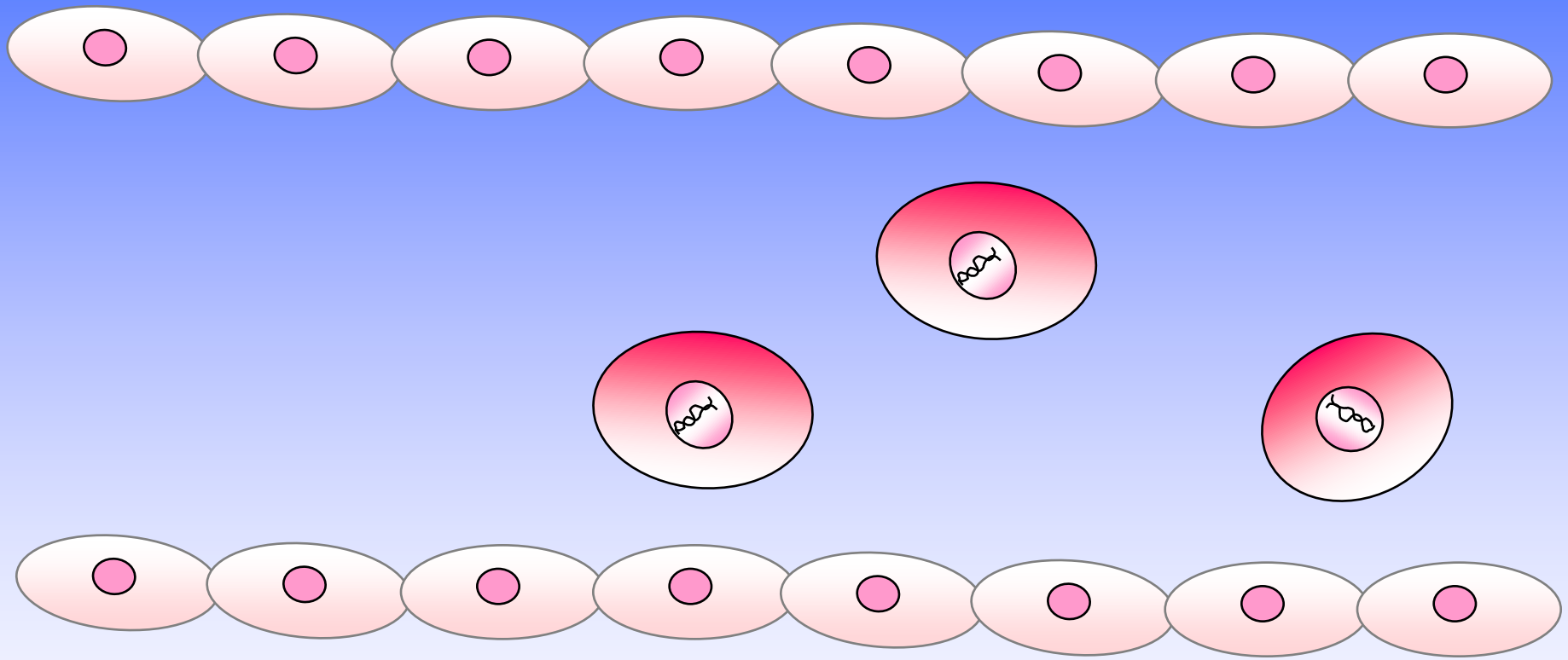
**Malignant cells transported from lymph node**

# DINO*M*IT-Metastasis

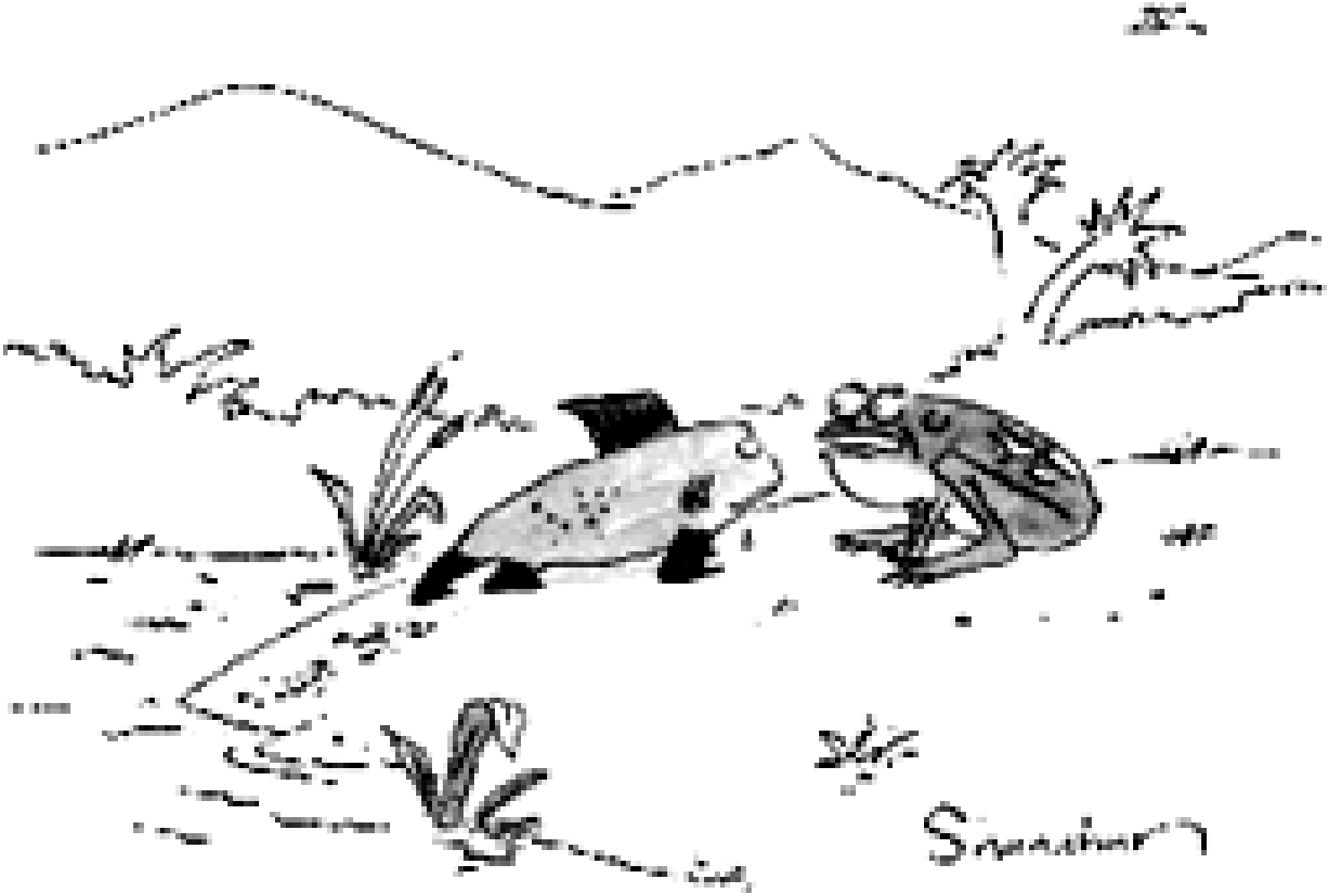


**Malignant cells transported from lymph node**

# DINO*M*IT-Metastasis



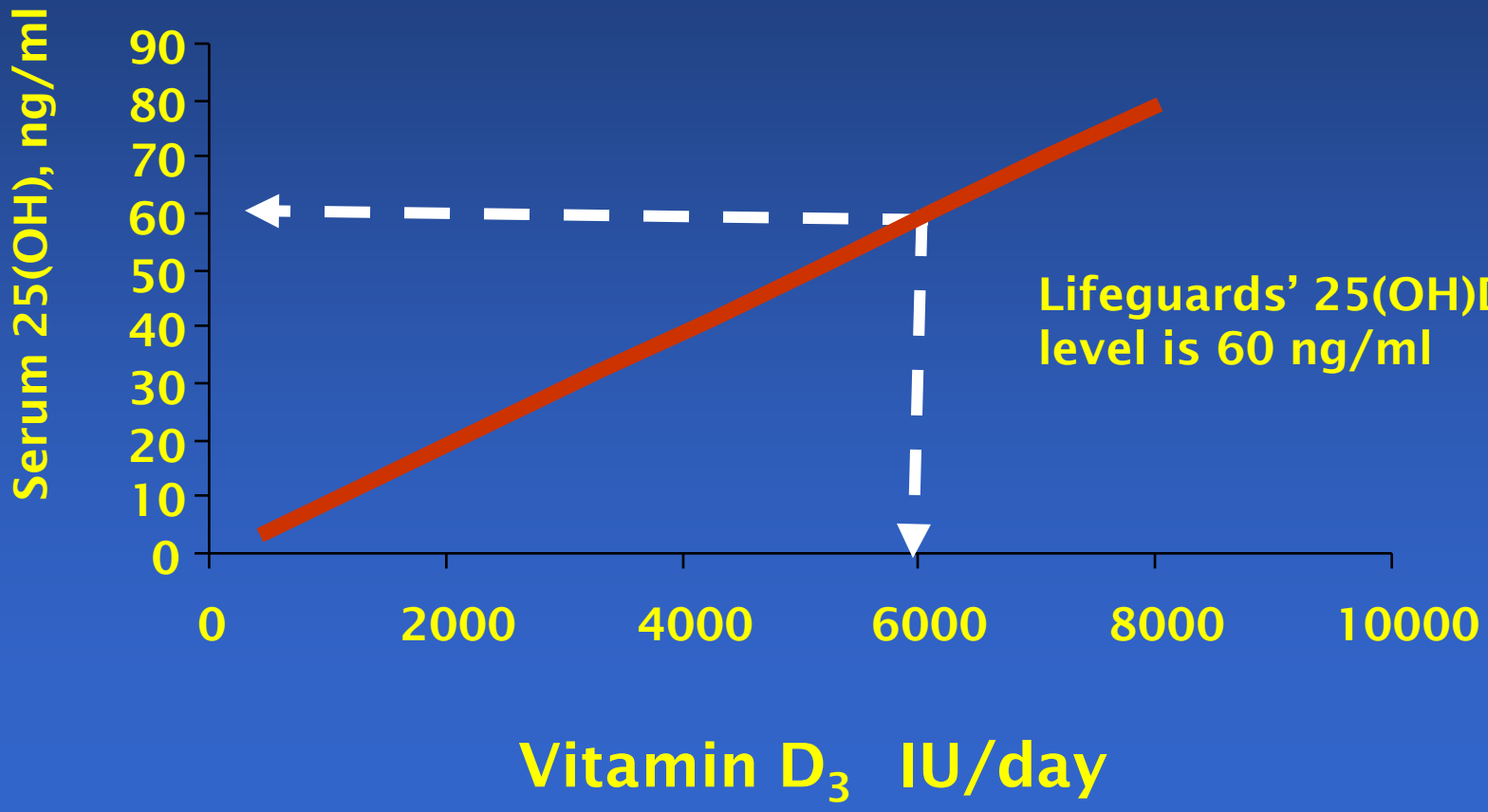
**Malignant cells transported from lymph node**

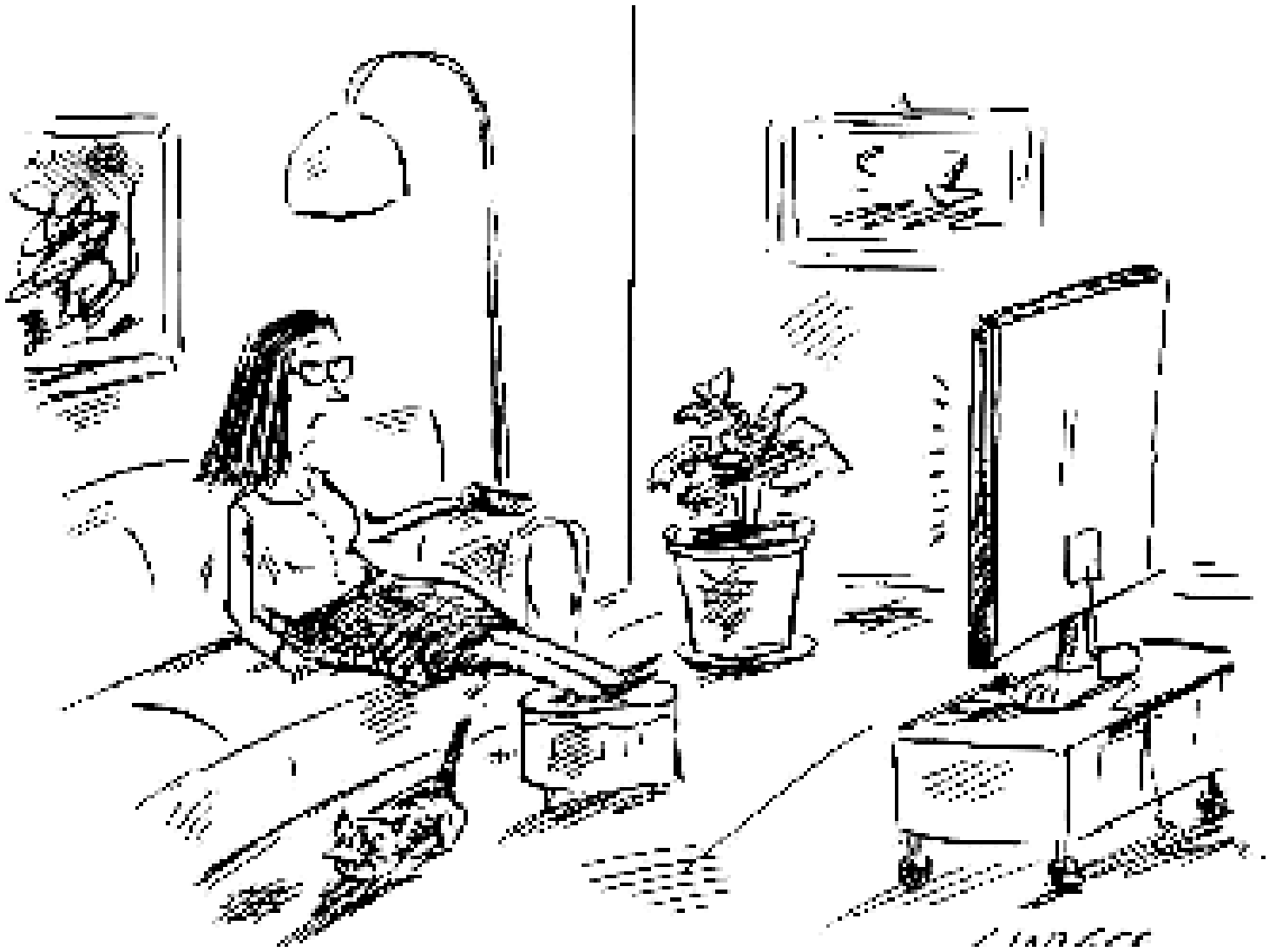


**The first thing you need to do is update your *CV***

# Serum 25(OH)D by oral intake of vitamin $D_3$

**Rule of thumb: Each 1000 IU of vitamin $D_3$ intake increases serum 25(OH)D by approximately 10 ng/ml**





**Ask your doctor if taking a pill to solve all your problems is right for you.**

# Vitamin D Status for Breast Cancer Prevention

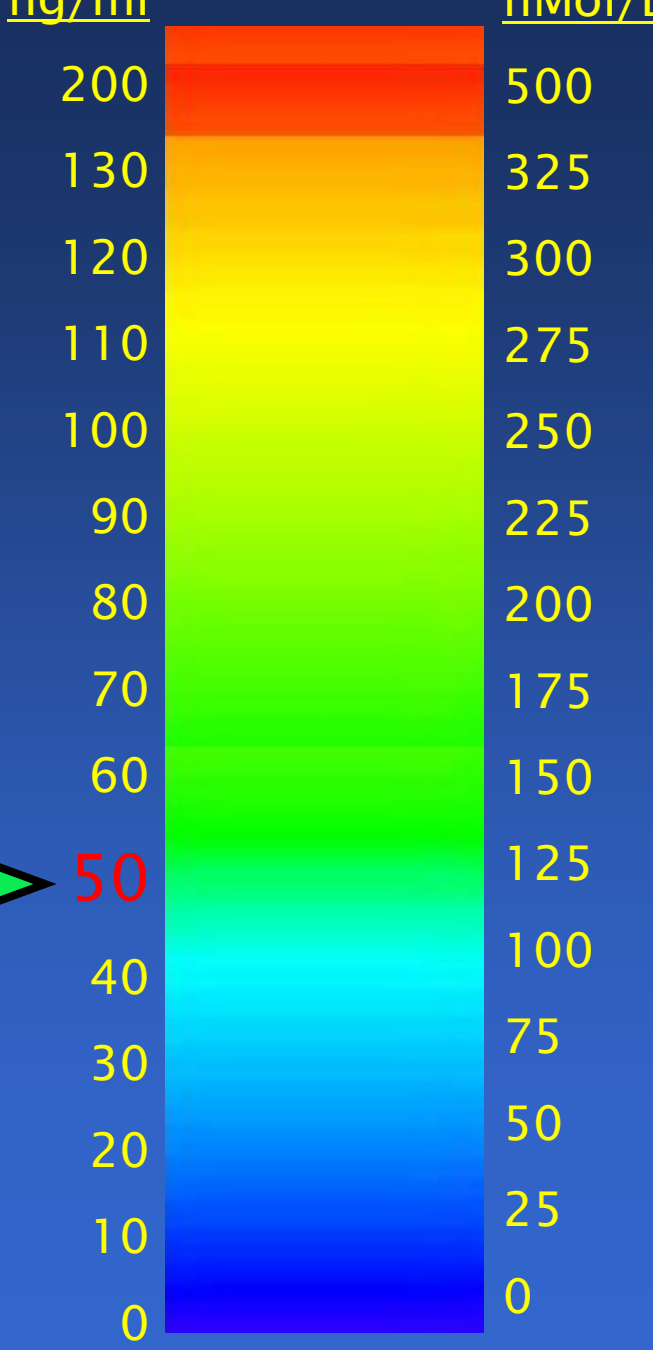
**Optimal serum 25 (OH) Vitamin D for breast cancer prevention?**

**People in sunny places have serum 25(OH)D levels of 54 to 90 ng/ml (1). Adults excrete 3,000-5,000 IU/day of vitamin D (2).**

**A good clinical target for breast cancer prevention:**

50 nanograms/ml

**Each 1000 IU of vitamin $D_3$ serum 25 (OH)D increases 10 ng/ml. (2).**



# Breast Cancer

**Vitamin $D_3$**:

Serum target, all ages…40-60 ng/ml

Oral intake……1,000-2,000 IU/day or as needed for above serum level

Recommend 6 cups/day of fluids (I500 ml) and 1000 mg/day of calcium, or as needed for bone density.

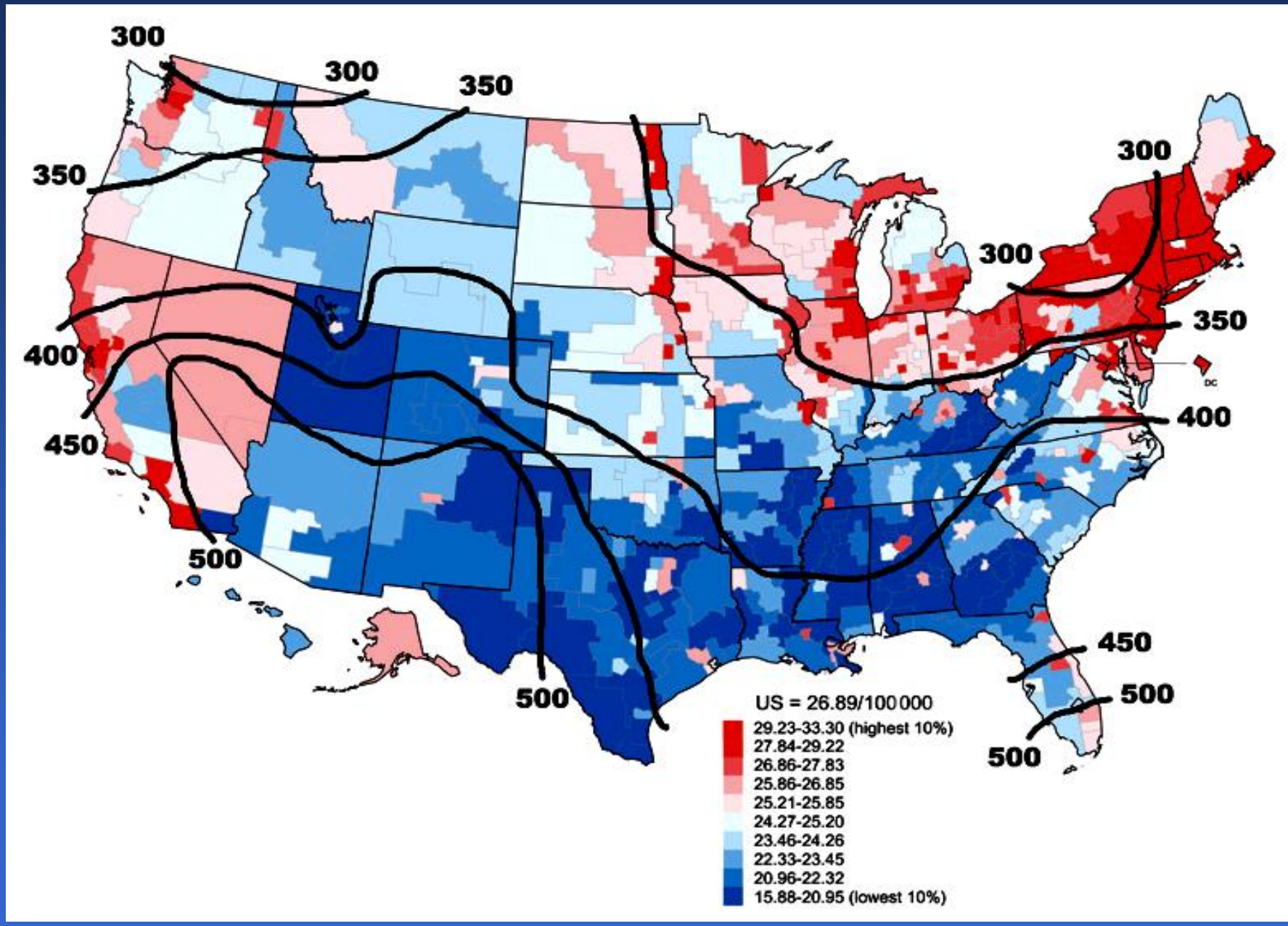
# Breast Cancer Patients

1. Draw blood for serum 25-hydroxyvitamin D, calcium and ionized calcium.
2. Start patients with breast adenocarcinoma on 2000 IU/day of vitamin $D_3$ and 1000 mg/day of calcium, unless hypercalcemic, regardless of other treatment.
3. Titrate vitamin $D_3$ intake upward to maintain 55-60 ng/ml 25-hydroxyvitamin $D_3$

# Breast Cancer Patients

1. Re-test serum 25-hydroxyvitamin D and calcium monthly.
2. For selected patients, consider suggesting not more than 10 minutes/day **outdoors near solar noon, weather allowing, with 40% skin exposure**, unless there is a history of skin cancer or photosensitivity. No sunscreen for 10 minutes. **Goal is is 0.75 minimal erythemal dose (MED)/day.**
3. Maintain fluid intake ( ≥1500 ml/day).

# Breast cancer mortality in the USA



# Footnotes

| | Source |
|---|---|
| 1 | Tangrea J, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control 1997;8:615–25. |
| 2 | Feskanich D, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004;13:1502– 8. |
| 3 | Wactawski-Wende J, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. New Engl J Med 2006; 354:684-96. |
| 4 | Gorham ED, et al. Optimal vitamin D status for colorectal cancer prevention: A Quantitative Meta Analysis. Am J Prev Med March 2007; 32:210-6. |
| 5 | Gorham ED, et al. Optimal vitamin D status for colorectal cancer prevention: A Quantitative Meta Analysis. Am J Prev Med March 2007; 32:210-6. |
| 6 | Freedman DM, Looker AC, Shih-Chen C, et al. Prospective study of serum vitamin D and cancer mortality in the United States. J Natl Cancer Inst 2007;99:1594-602. |
| 6 | Ng K, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, Giovannucci EL, Fuchs CS. Circulating 25-hydroxyvitamin D levels and survival in patients with colorectal cancer J Clin Oncol 2008; 26: 2984-91. |
| 7 | Data from: Lowe LC, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer. 2005;41:1164-9. |
| 8 | P J Goodwin, et al. Vitamin D deficiency is common at breast cancer diagnosis and is associated with a significantly<br>higher risk of distant recurrence and death in a prospective cohort study. American Society of Clinical Oncology Annual<br>Meeting, Chicago, Illinois, May 30-June 3, 2008. Abstract number: 08-AB-31397-ASCOAM. |
| 9 | Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007;85:1586-91. |

# Footnotes

| | |
|---|---|
| 10 | Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007;85:1586-91. |
| 11 | Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007;85:1586-91. |
| 12 | 1. Barger-Lux et al. Osteoporosis Intl 1998; 8: 222-30;<br>2. Haddad and Chyu. Clin Endocrinol Metab 1971; 33: 992-5. |
| 13 | 1. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr. 2005;135:317-22<br><br>2. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr. 2003;77:204-10. |