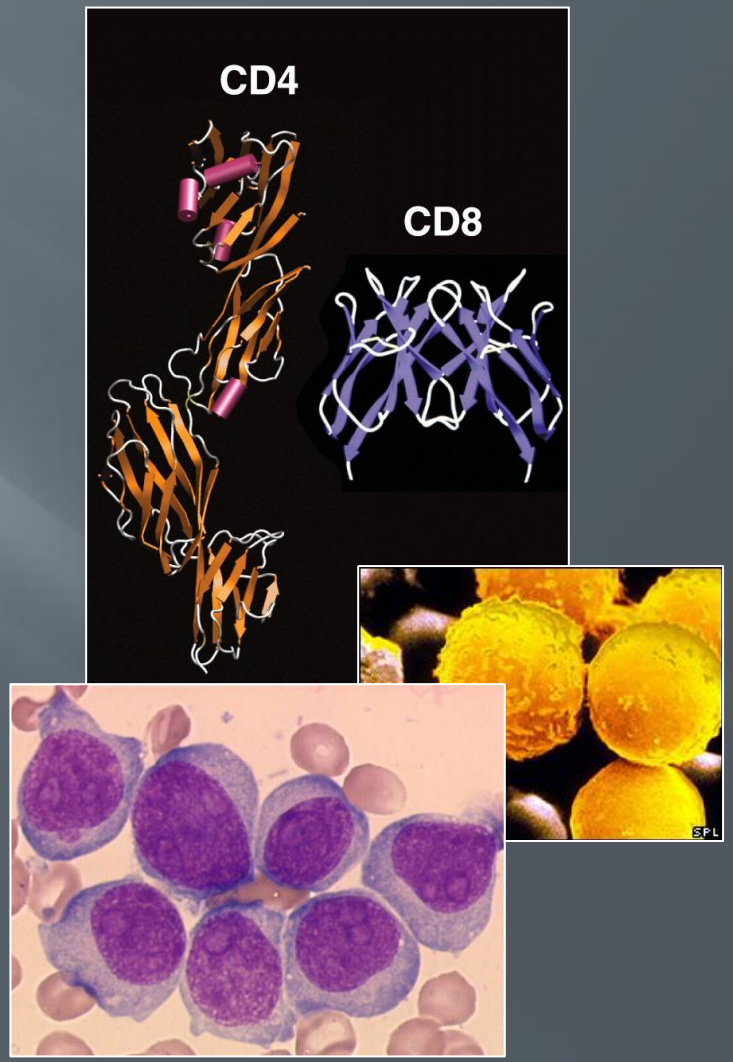
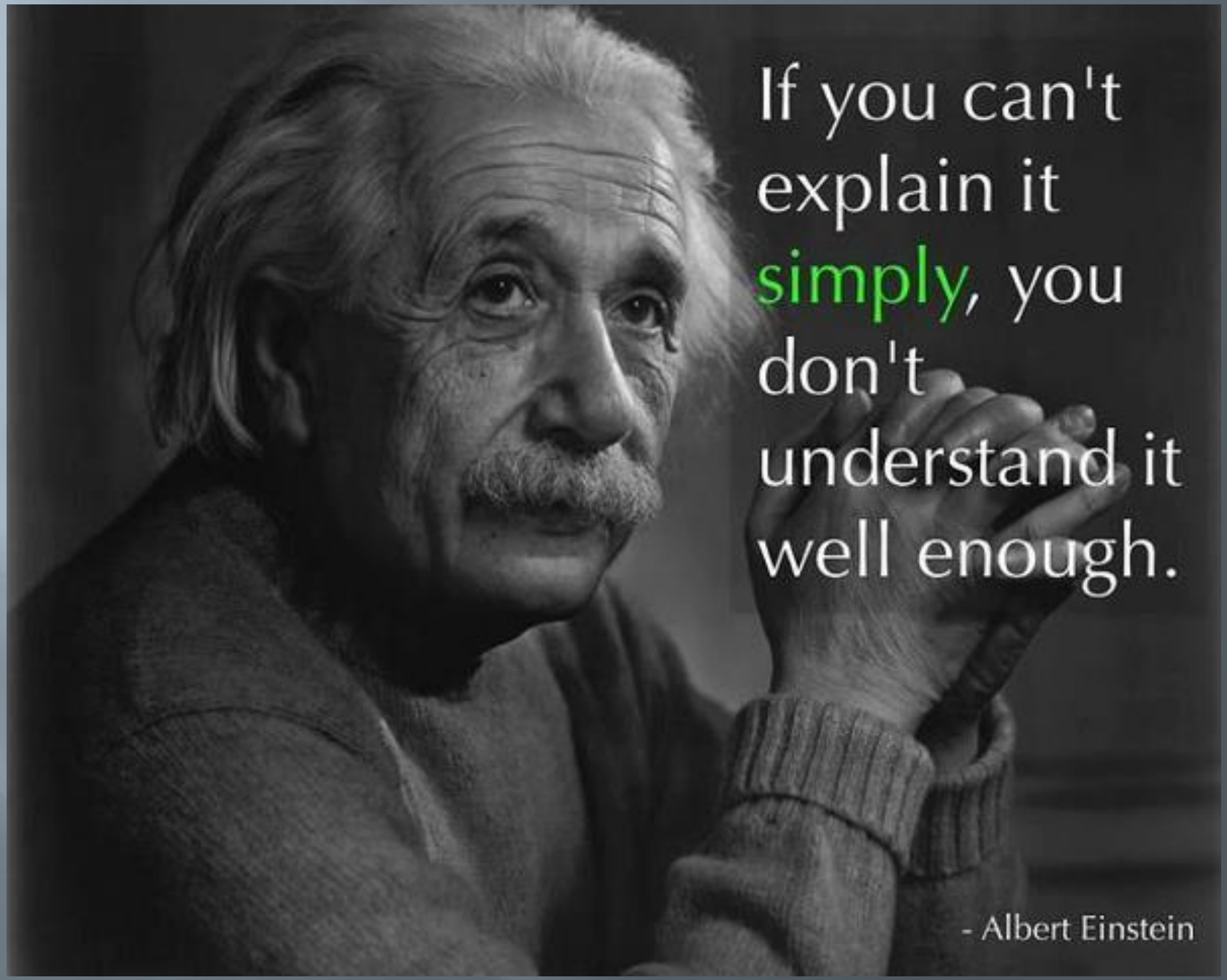
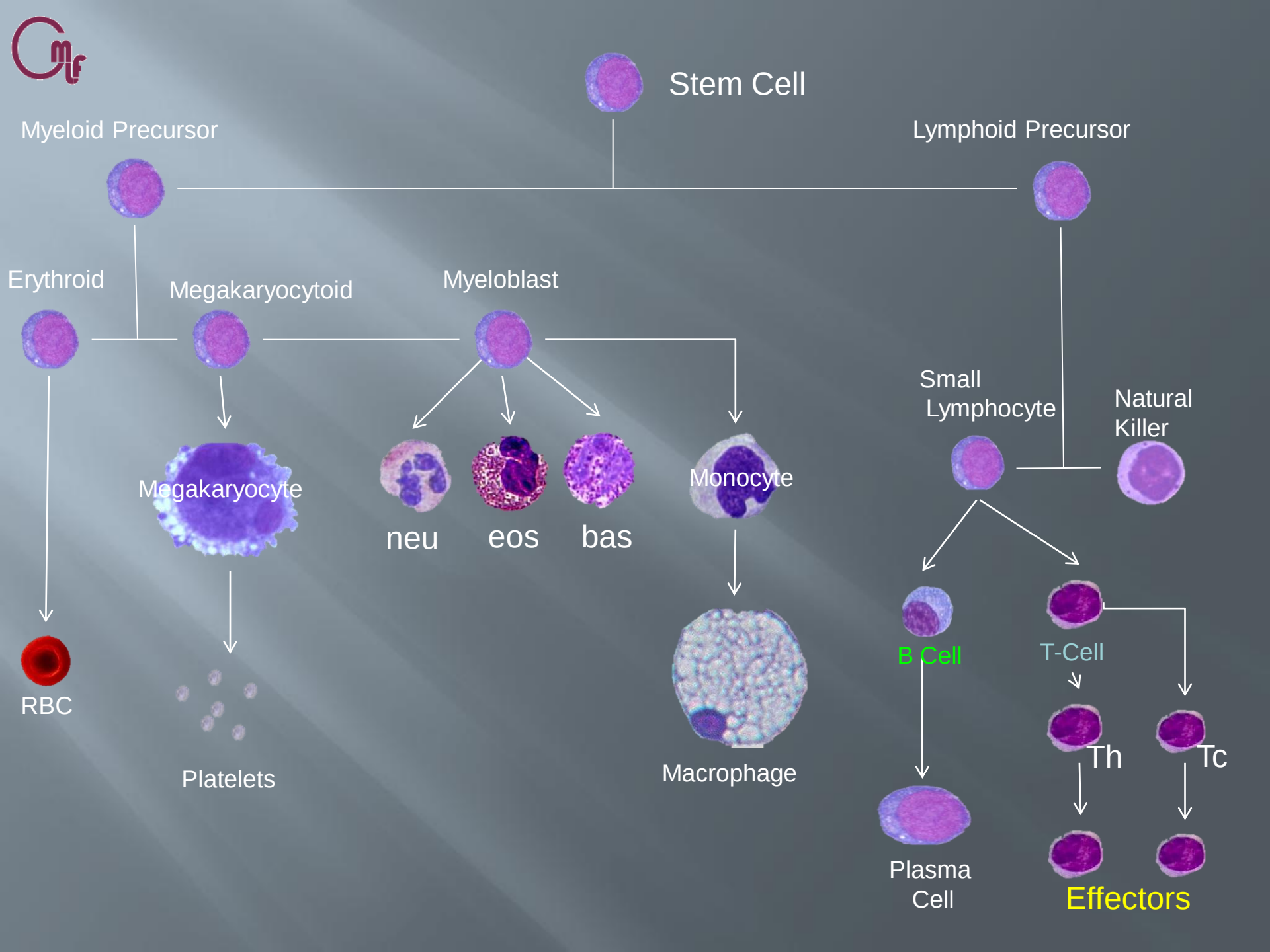
Leukaemia, Lymphoma and Flow Cytometry



Wayne Labastide

Caribbean Med Labs Foundation (CMLF)


If you can't explain it simply, you don't understand it well enough.
- Albert Einstein


Stem Cell
Myeloid Precursor
Lymphoid Precursor
Erythroid
Megakaryocytoid
Myeloblast
Small Lymphocyte
Natural Killer
Megakaryocyte
neu
eos
bas
Monocyte
RBC
Platelets
Macrophage
B Cell
T-Cell
Th
Tc
Plasma Cell
Effectors

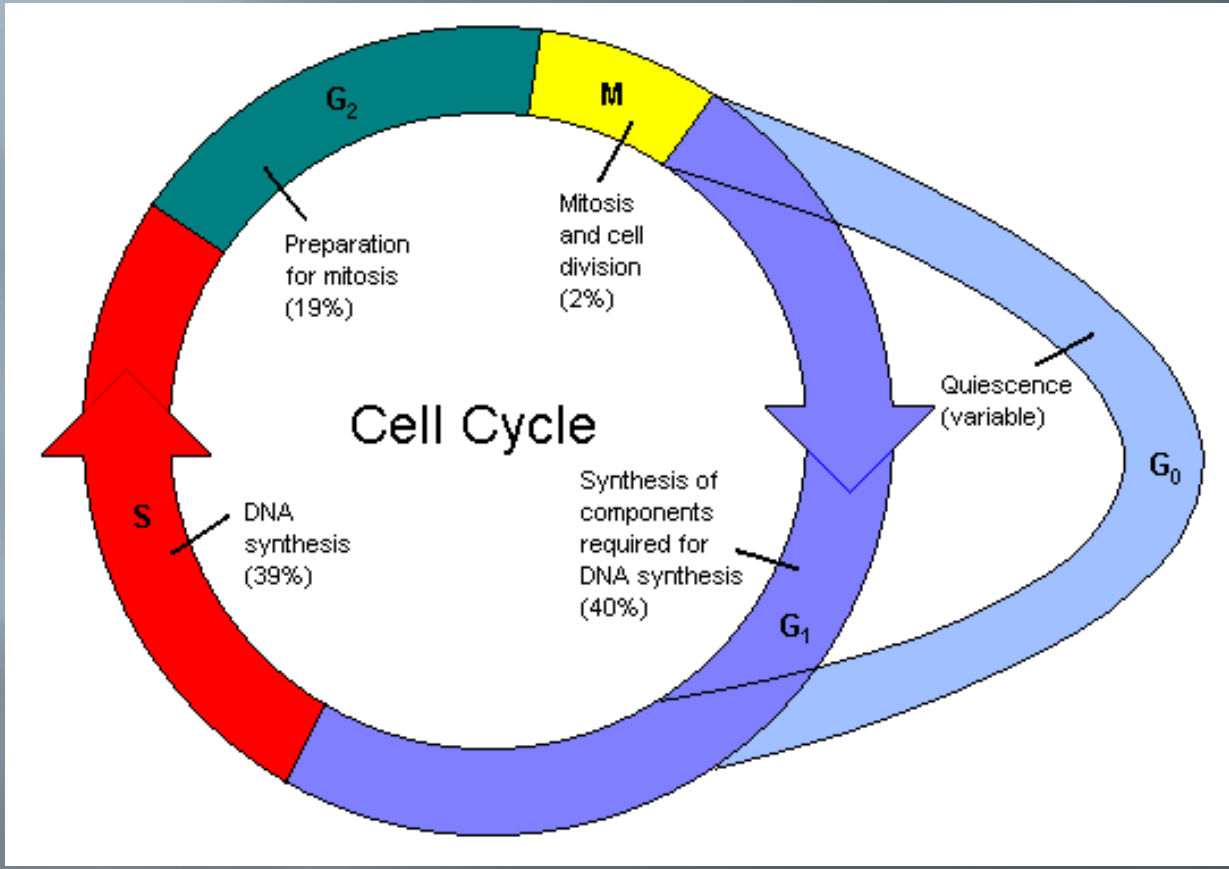
What is malignancy, exactly?

- A genetic error can take place at any stage of this process, causing a malignant transformation.

Fact or Fiction?

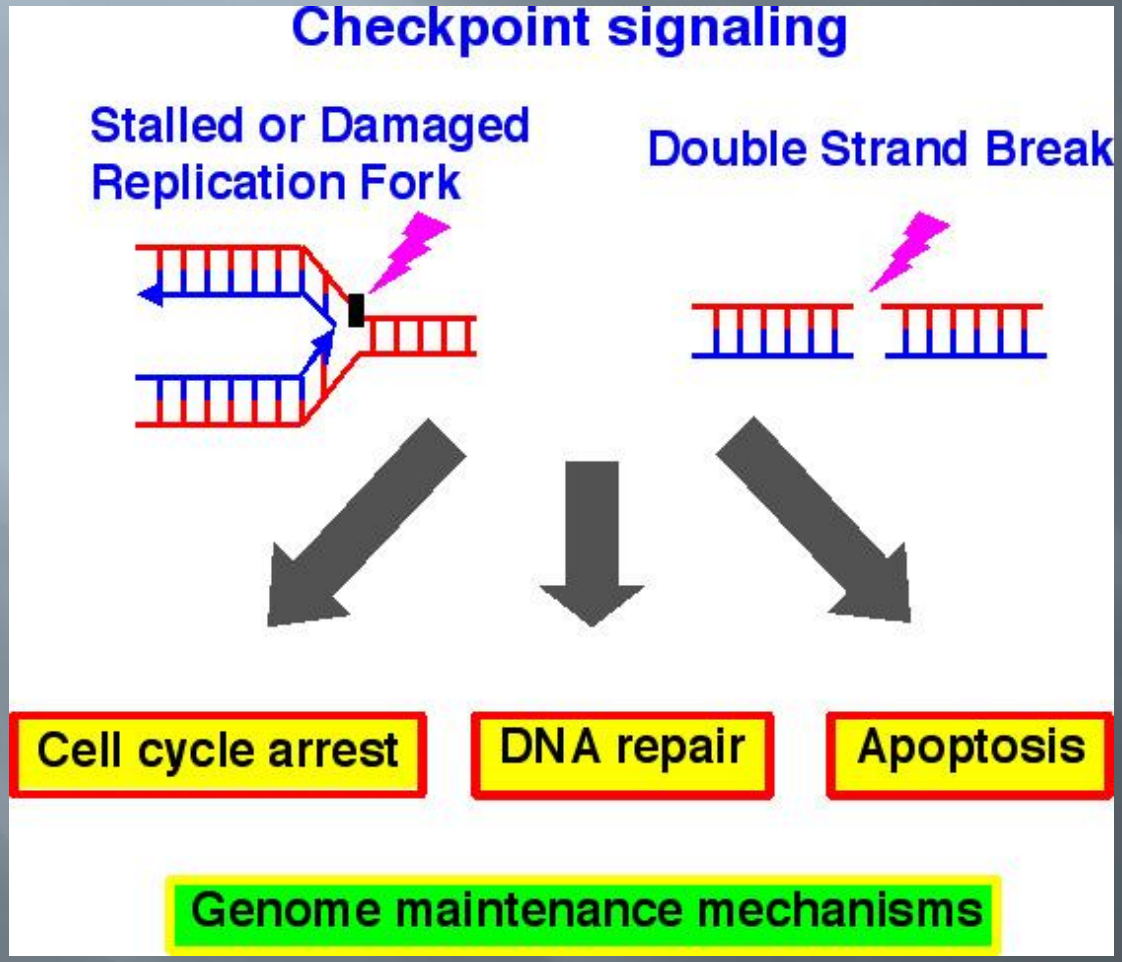
- Malignant cells cycle faster than their normal counterparts

Malignant cells have a growth advantage because they never go into G_0



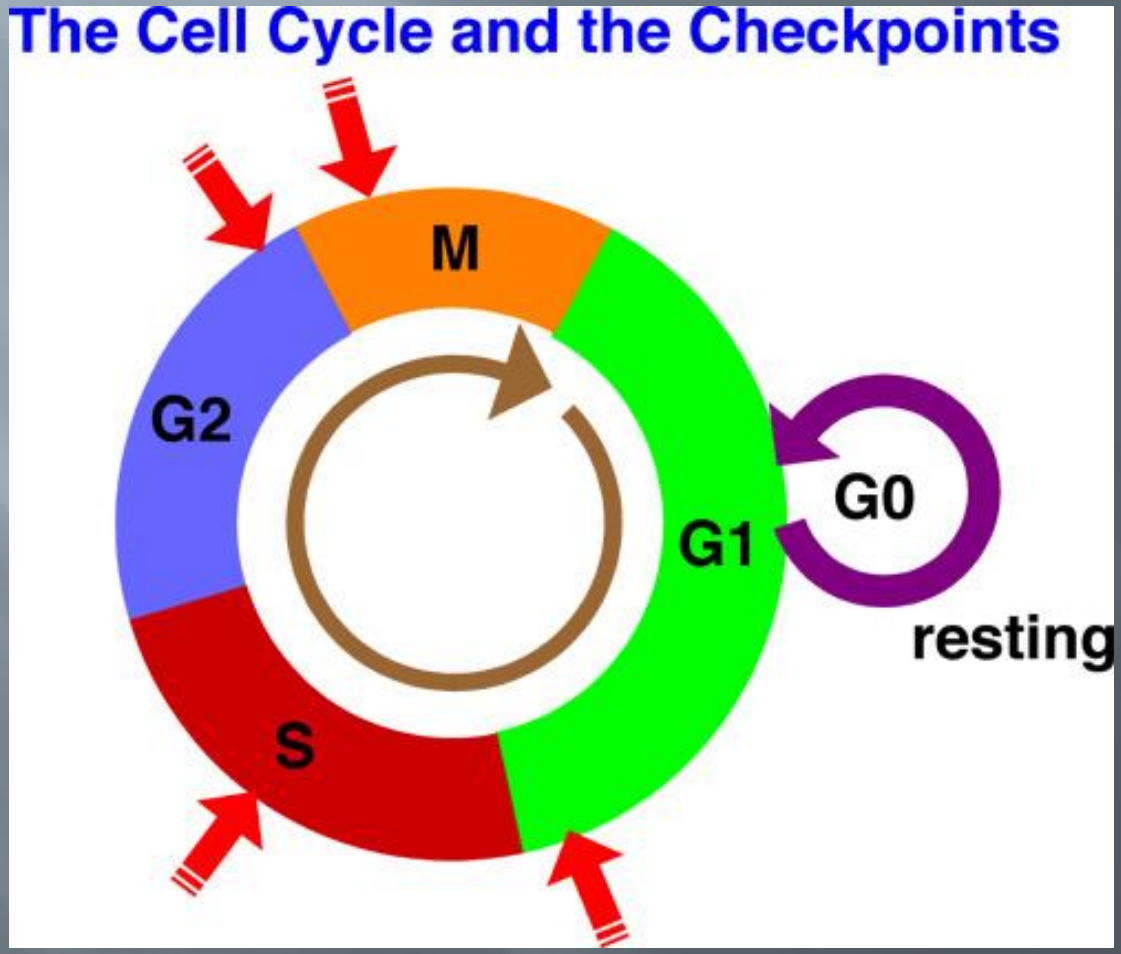
- Their cycle times are the same as their normal counterparts

There are natural checkpoints in the cell cycle



- If all is not well at each checkpoint, the cell goes into apoptosis

Anti-cancer drugs take advantage of the natural checkpoints



What causes malignant proliferation?

- Oncogene activation
  - Chromosomal translocations
  - Viruses

- Translocation:
  - A genetic fusion of part of a chromosome with another chromosome
- Oncogene:
  - A cellular growth-regulation gene that causes cancer when its function or expression is disrupted

- Malignant lymphocyte tumours often carry chromosomal translocations that join immunoglobulin loci to genes that regulate cell growth

- Burkitt's Lymphoma: translation of the oncogene Myc from chromosome 8 to the Ig gene on Chromosome 14

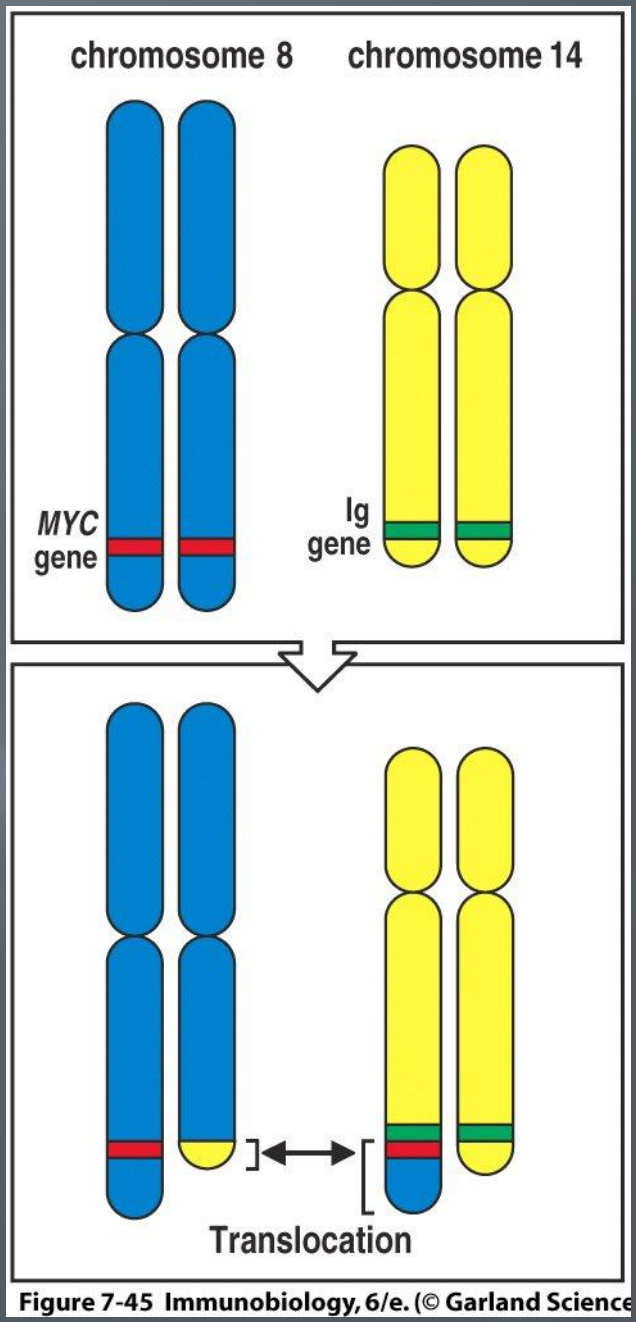


Figure 7-45 Immunobiology, 6/e. (© Garland Science

- Immunoglobulin and T-cell receptor genes are sites of breakage of DNA during gene rearrangement, isotype switching and somatic hypermutation.
- They are especially likely sites for translocation
- Translocation of an oncogene with an immunoglobulin gene enhancer or promoter may result in permanent activation of the oncogene

Viruses

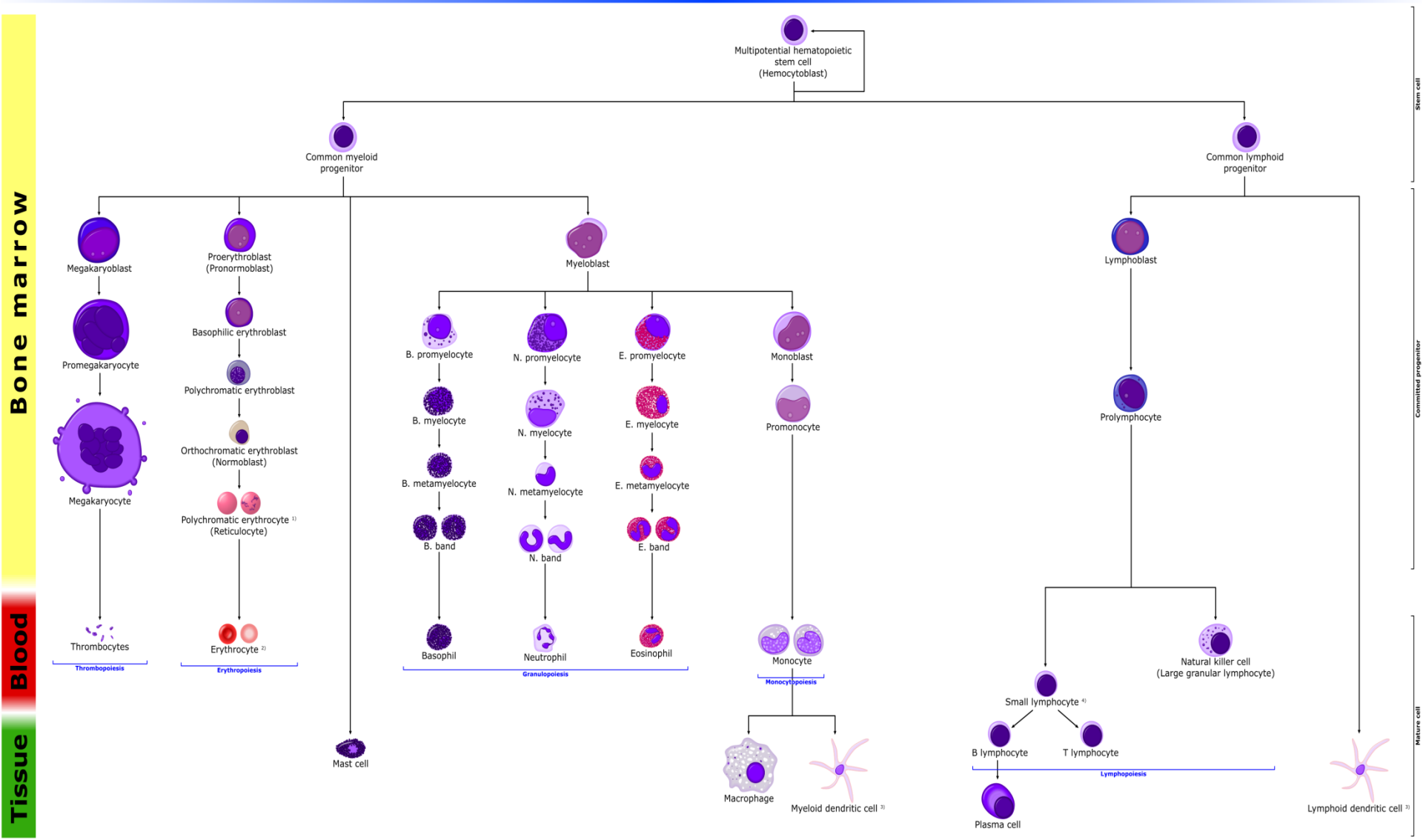
- Herpes viruses and retroviruses generally infect cells without killing them.
- Stimulation of uncontrolled growth is beneficial to the viruses
- Viral promoter sequences can also activate oncogenes

Two important changes

- The transformed cell proliferates without regulation.
- The transformed cell stops differentiating.

- (what is the resulting effect?)

Hematopoiesis in humans



Notes

- Approximate scale information: 10 µm
- The morphological characteristics of the hematopoietic cells are shown as seen in a *Wright's* stain, *May-Giemsa* stain or *May-Grünwald-Giemsa* stain. Alternative names of certain cells are indicated between parentheses.
- Certain cells may have more than one characteristic appearance. In these cases, more than one represenation of the same cell has been included.
- Together, the monocyte and the lymphocytes comprise the *agranulocytes*, as opposed to the *granulocytes* (basophil, neutrophil and eosinophil) that are produced during granulopoiesis.
- *B.*, *N.* and *E.* stand for *Basophilic*, *Neutrophilic* and *Eosinophilic*, respectively. As in Basophilic promyelocyte.

1) The polychromatic erythrocyte (reticulocyte) at the right shows its characteristic appearance when stained with methylene blue or Azure B.

2) The erythrocyte at the right is a more accurate representation of what it looks like in reality when viewed through a microscope.

3) Other cells that arise from the monocyte: osteoclast, microglia (central nervous system), Langerhans cell (epidermis), Kupffer cell (liver).

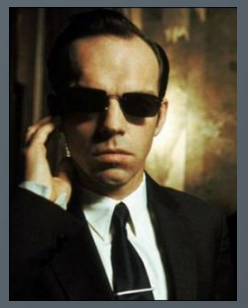
4) For clarity, the T and B lymphocyte are split to better indicate that the plasma cell arises from the B-cell. Note that there is no difference in the appearance of B- and T-cells unless specific staining is applied.

Effect of transformation:

- Accumulation of cells blocked at a certain stage of differentiation.

The nature of malignancy:

- Transformation began with one cell.



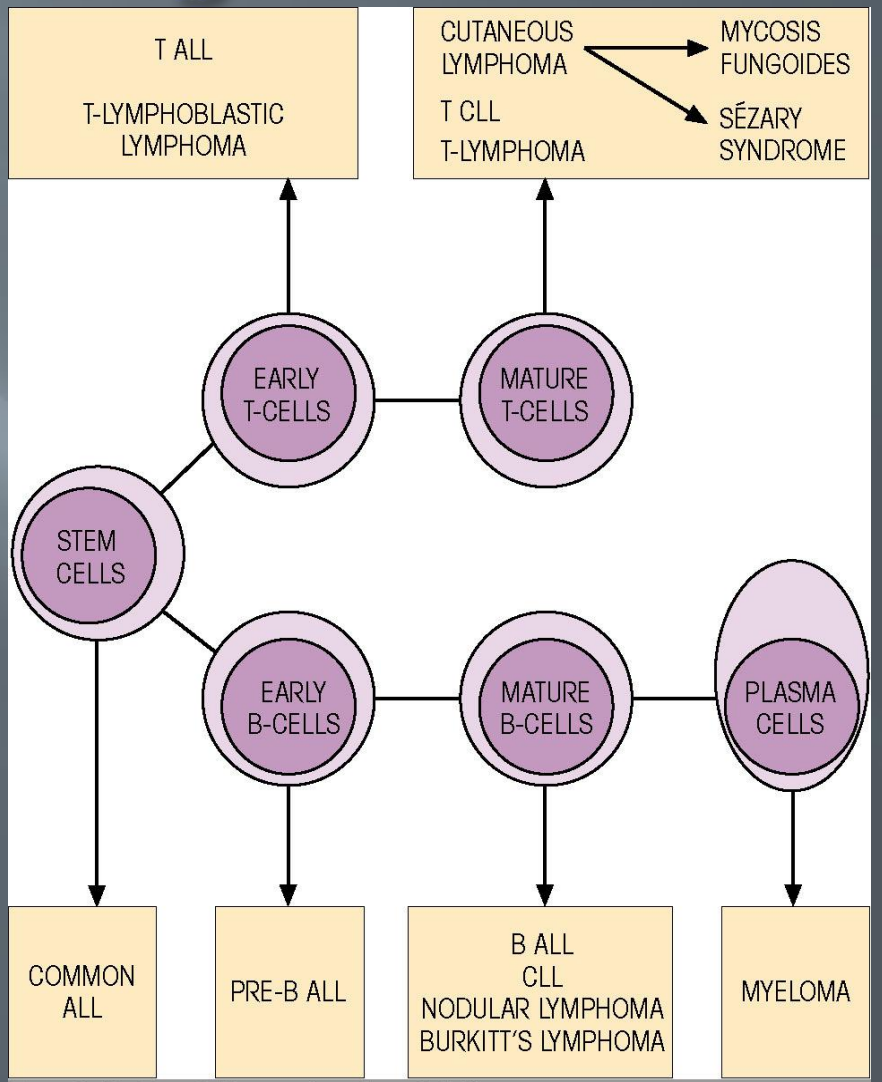
- All the diseased cells are of the same clone
- Malignancy is characterised by a monoclonal proliferation

Value of Flow Cytometry

- Identifies the Lineage
- Identifies the stage of differentiation
- Demonstrates monoclonality

Lymphoid malignancies

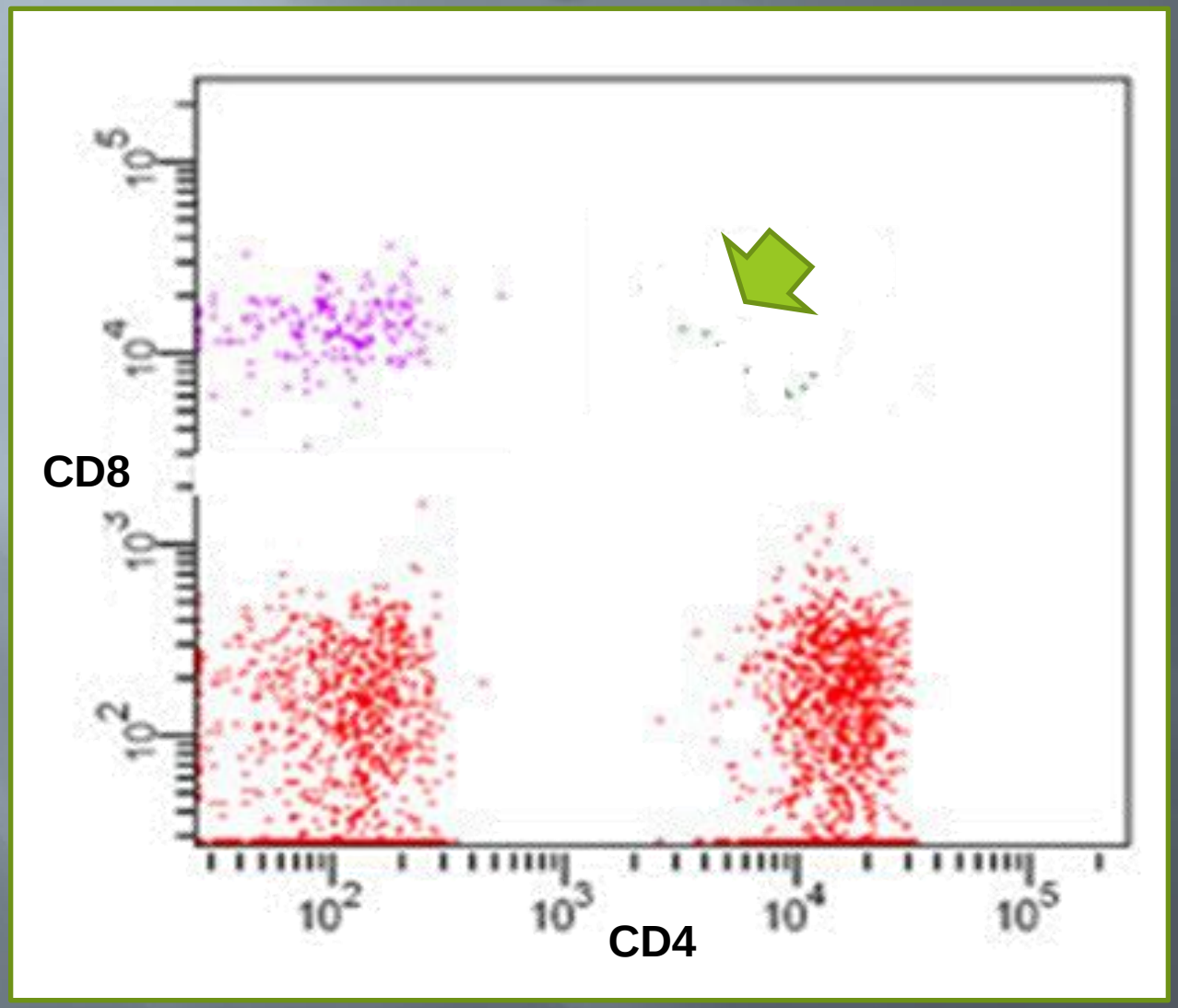
- Lymphoid tumours represent monoclonal outgrowths of normal cell populations
- Lymphoid malignancies have helped us understand normal lymphocyte development



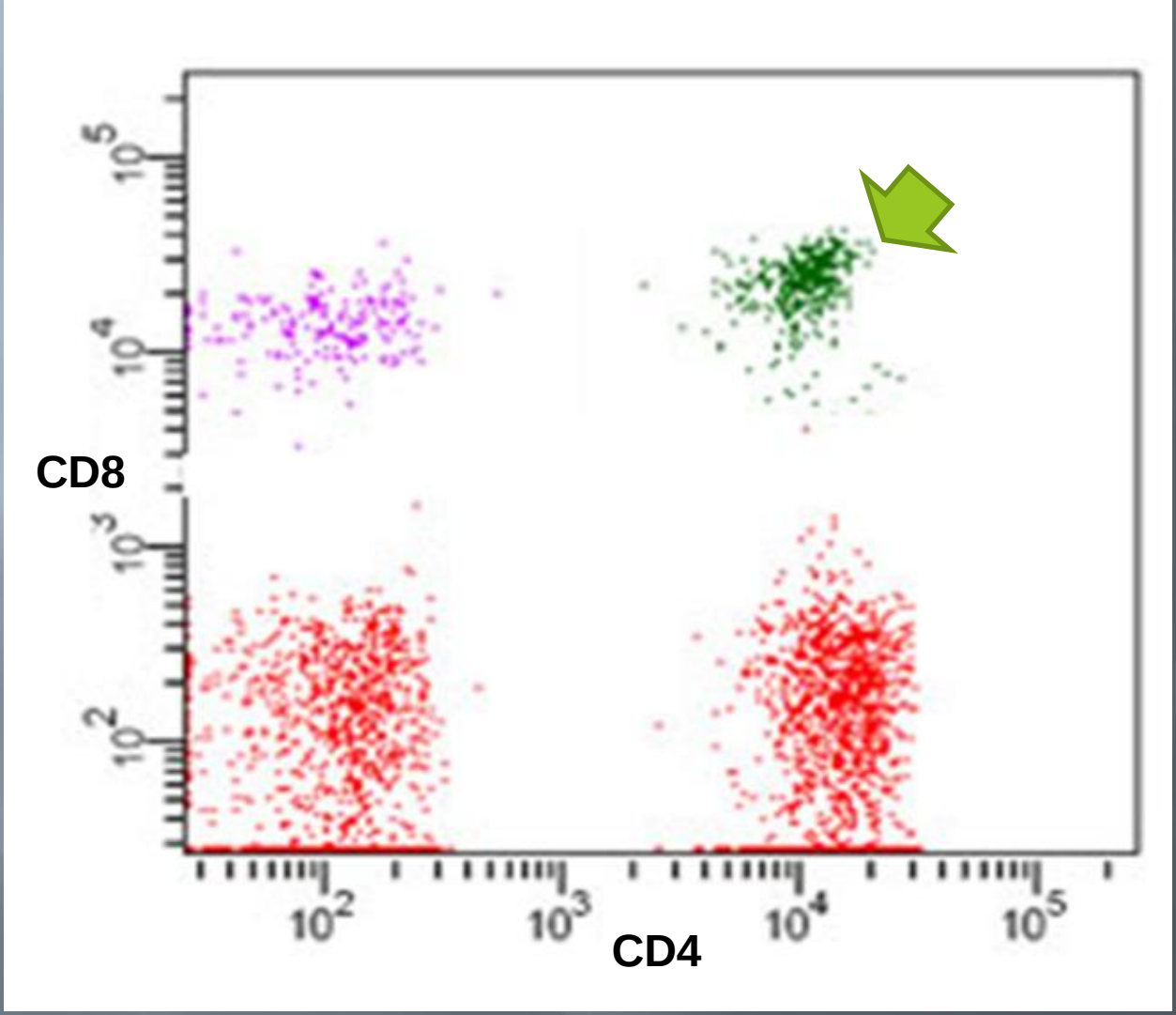
From: *Roitt's Essential Immunology* Eleventh Edition

- B-cell malignancies each have a corresponding normal B-cell equivalent
- B-cell malignancies can occur at almost every stage of B-cell development

Rethinking the "Rare Event"



The no-longer-Rare Event



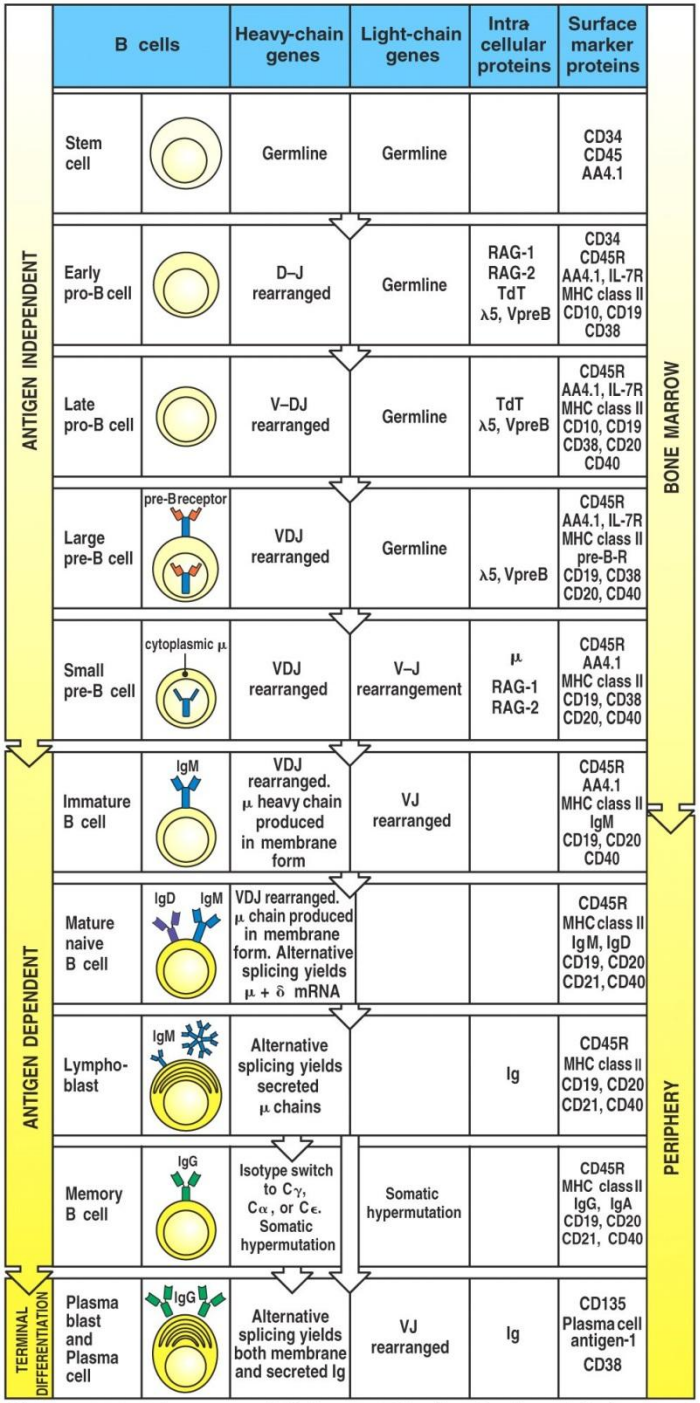


Figure 7-46 Immunobiology, 6/e. (© Garland Science 2005)

- Markers and stages of Normal B-cell development

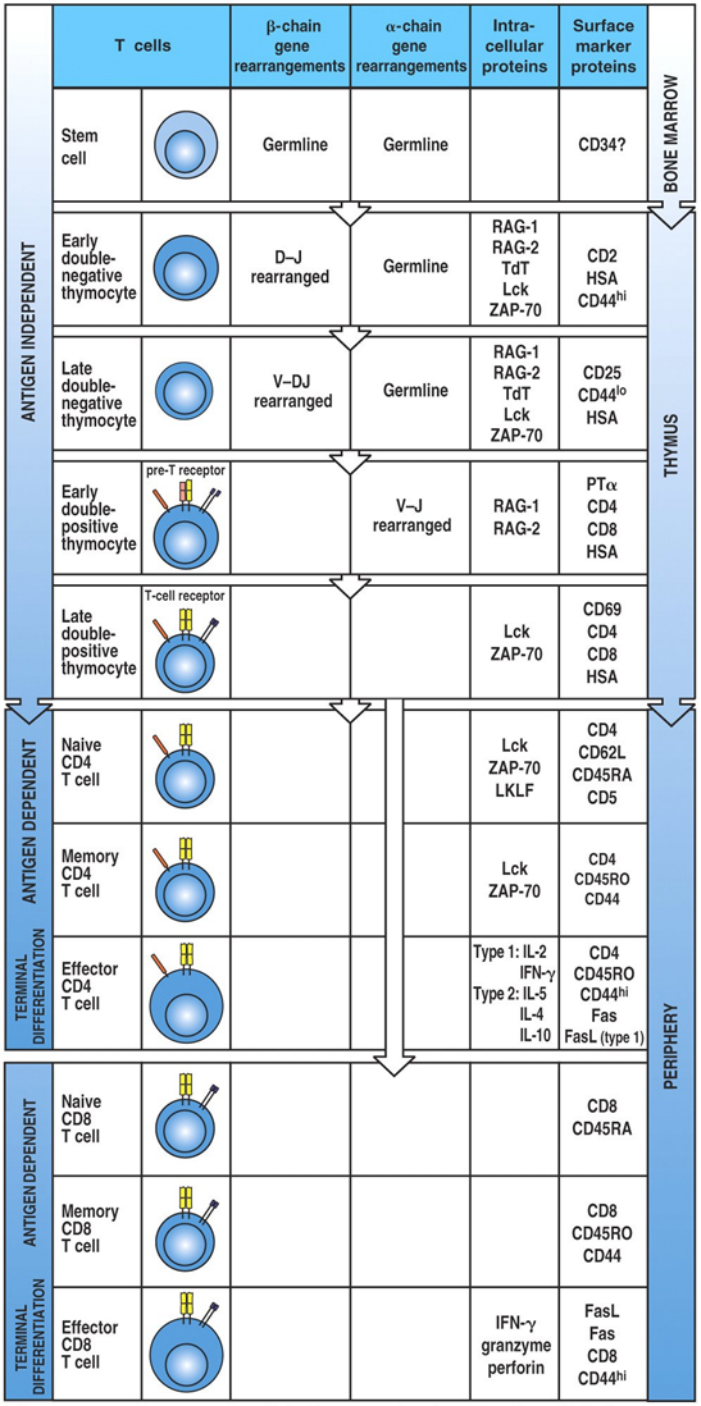


Figure 7-47 Immunobiology, 6/e. (© Garland Science 2005)

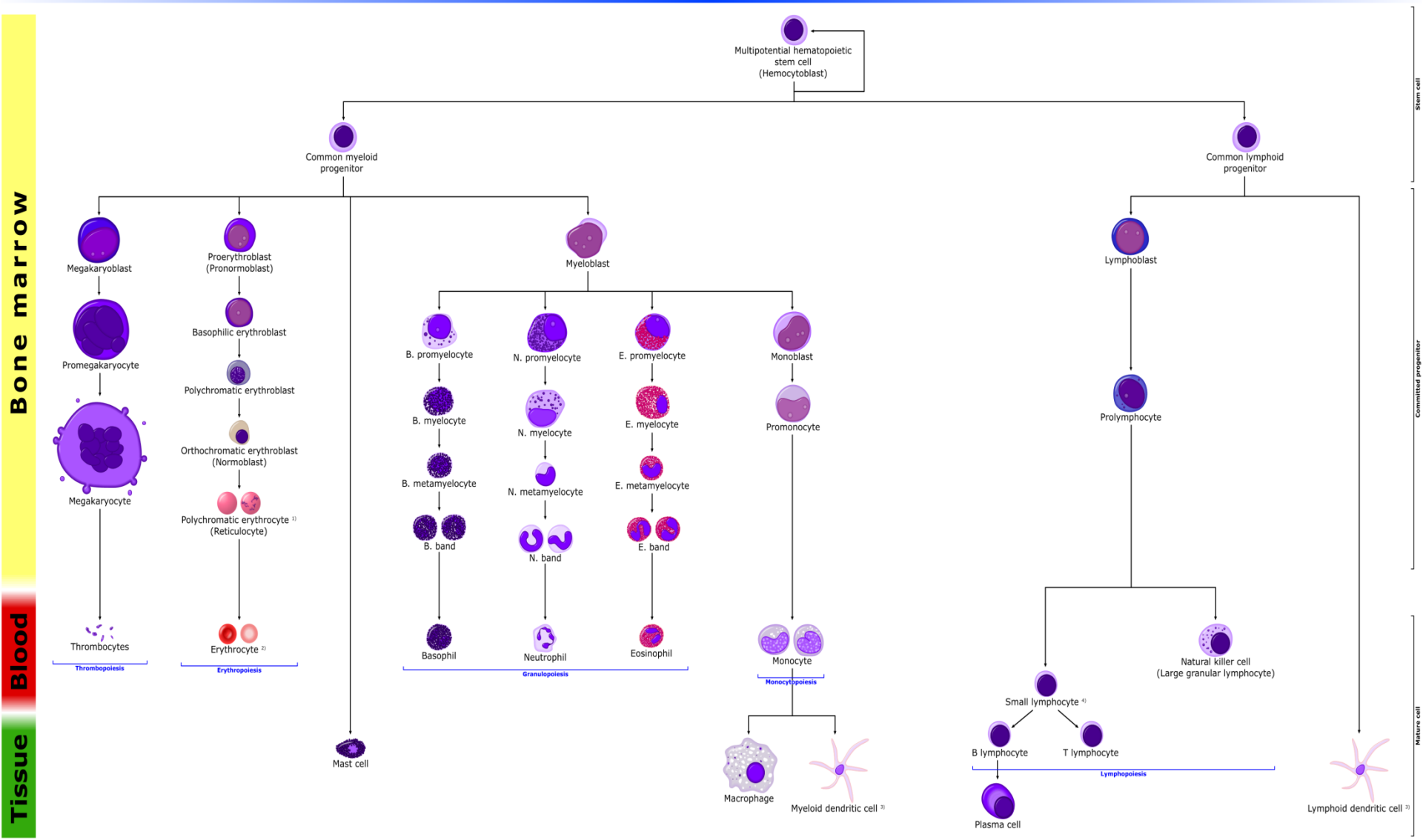
- Markers and stages of Normal T-cell development

Lymphoid malignancies

- Tumours retain many of the characteristics of the cell types from which they were derived
  - Cell surface markers
  - Homing properties
  - Gene rearrangements
  - Secreted products

- Identification of the lineage and developmental stage of a lymphoid tumour helps us to:
  - understand the disease presentation
  - develop strategies for therapy

Hematopoiesis in humans



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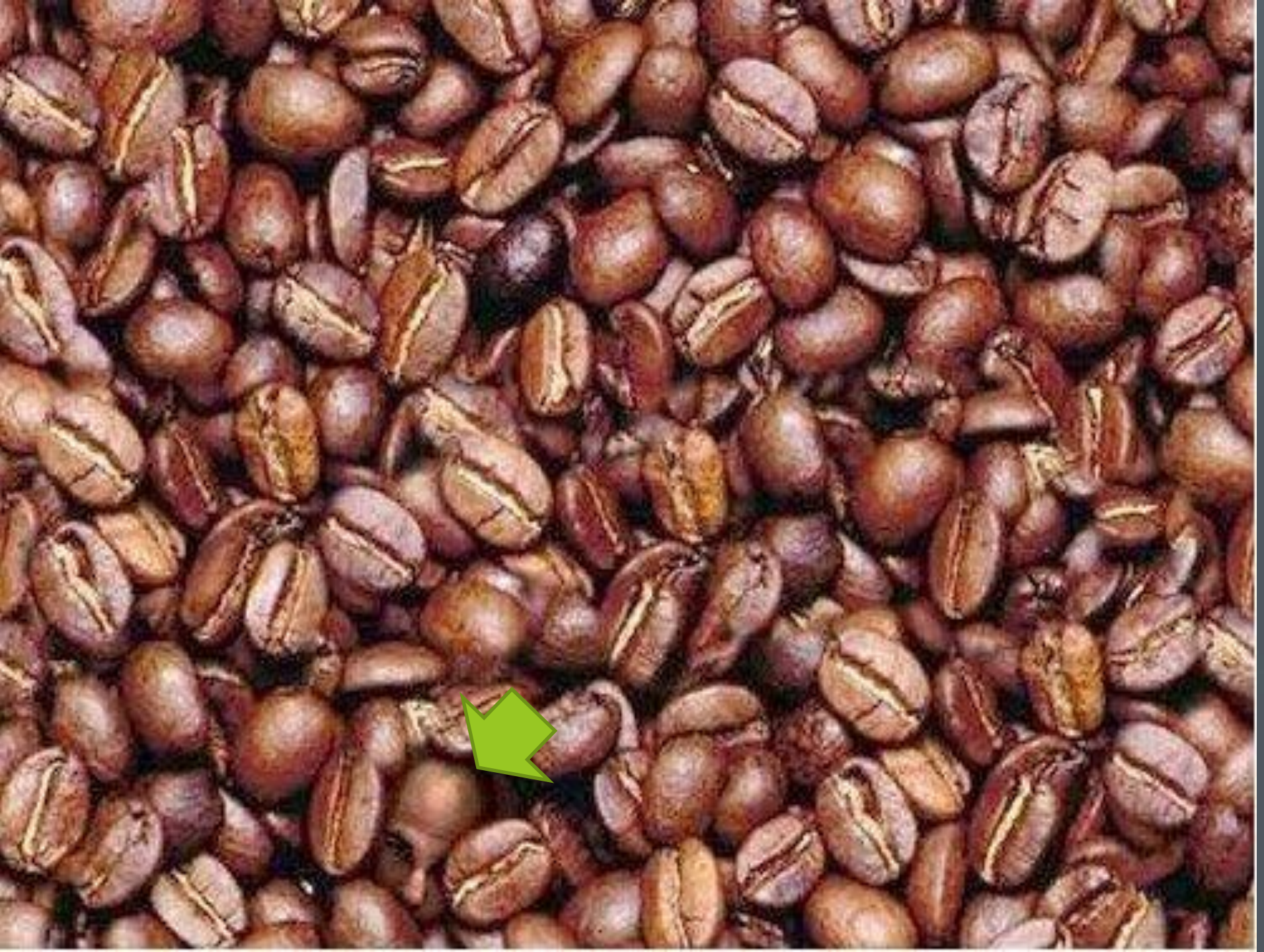
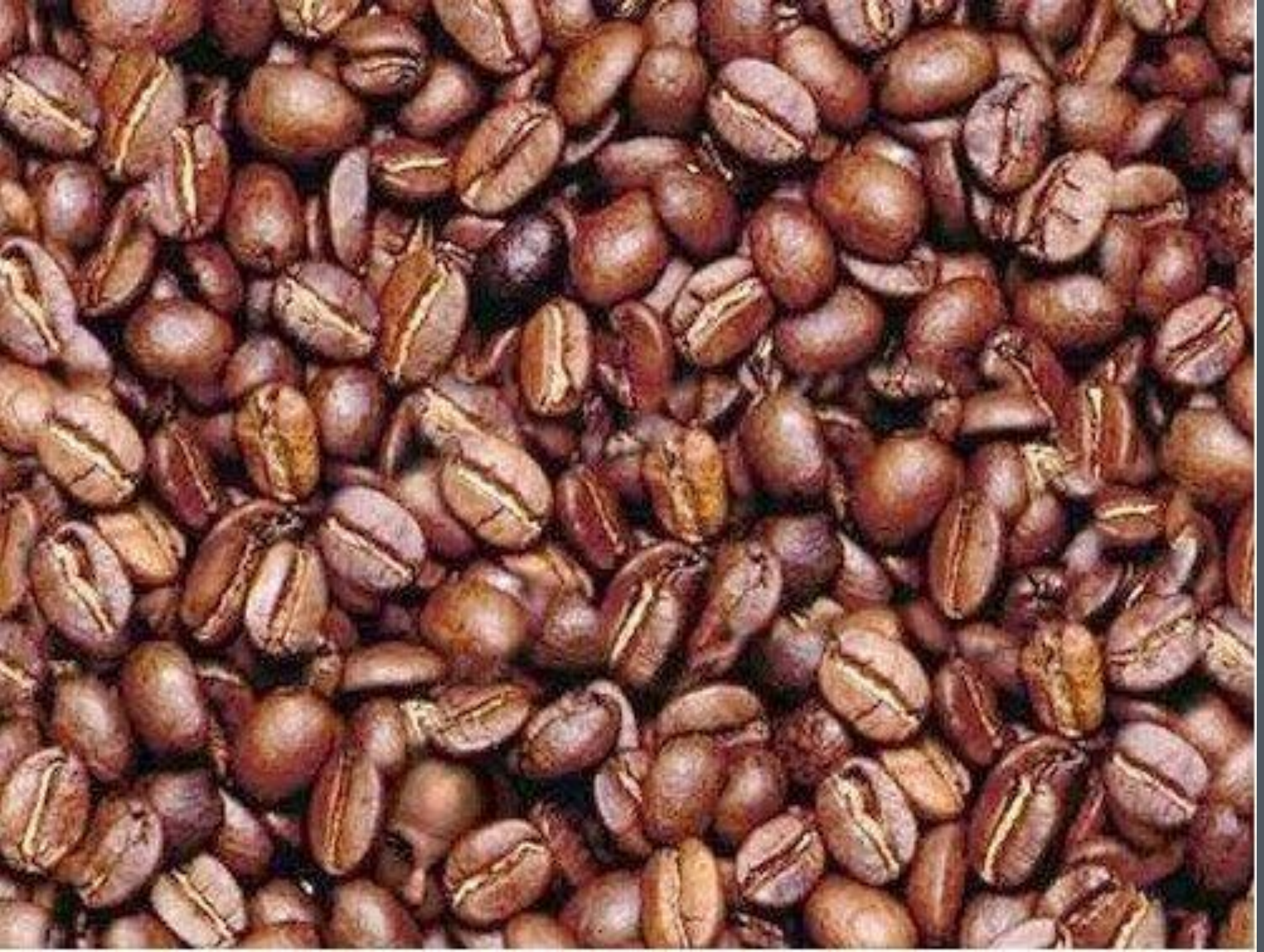
Monitoring Minimal Residual Disease (MRD)

- Rediscovering the "rare event"
- …once we know what we are looking for, it's not so difficult to find it.





Summary:

- The tumour is monoclonal
- The cells are out of (growth) control
- The cells have stopped differentiating
- Malignant cells accumulate at the stage of blockage
- This makes them visible to flow cytometry

Summary:

- Flow tells us the lineage and stage of differentiation
- Malignant cells generally keep their phenotypes and behaviours
- These behaviours influence the clinical presentation
- Knowing the lineage and stage helps determine prognosis and strategy

Understanding the disease gives us the power to fight back.