

Immunology (Day 13)

Polyclonal vs. monoclonal antibodies

	Polyclonal	Monoclonal
1. Specificity	multiple specificities	single specificity
2. B cell	multiple clones	single clone
3. Reproducibility	x	✓
4. Volume	x	✓
5. Isotype	Cross-reactive	Defined (x crossreactivity)

Polyclonal antibodies → localisation, phagocytosis, complement activation

Hybridoma Technology

- for research, monoclonal antibodies are preferable
- fusing normal activated antibody-producing B cell with myeloma cell → hybridoma cell

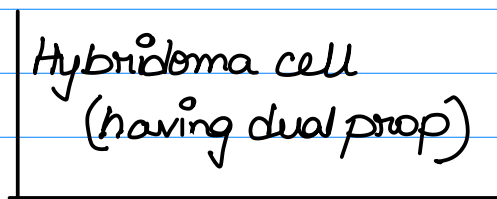
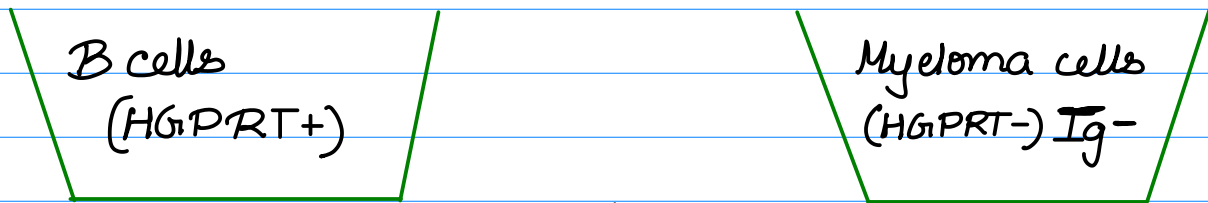
↪ immortal growth properties of myeloma cell + secretes antibody by B cell

- **hybridoma cells** secrete large quantities of monoclonal antibodies

Formation of hybridoma technology

Injection of desired antigen into mice

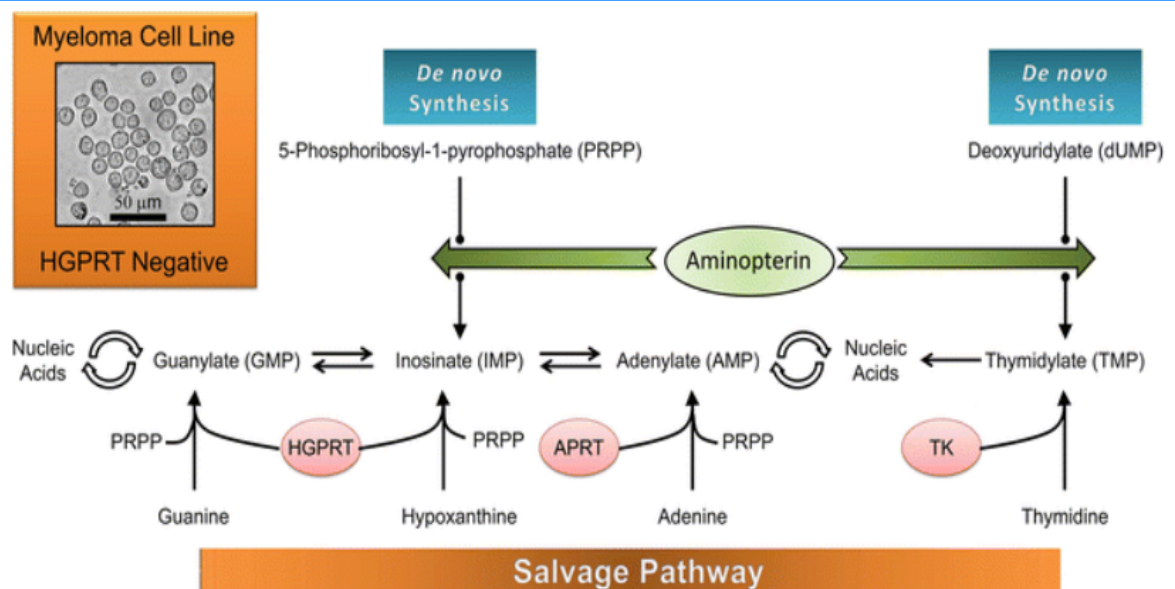
↓
Harvesting of mouse spleen to obtain B-cells



Culture in HAT medium → selection for positive cells

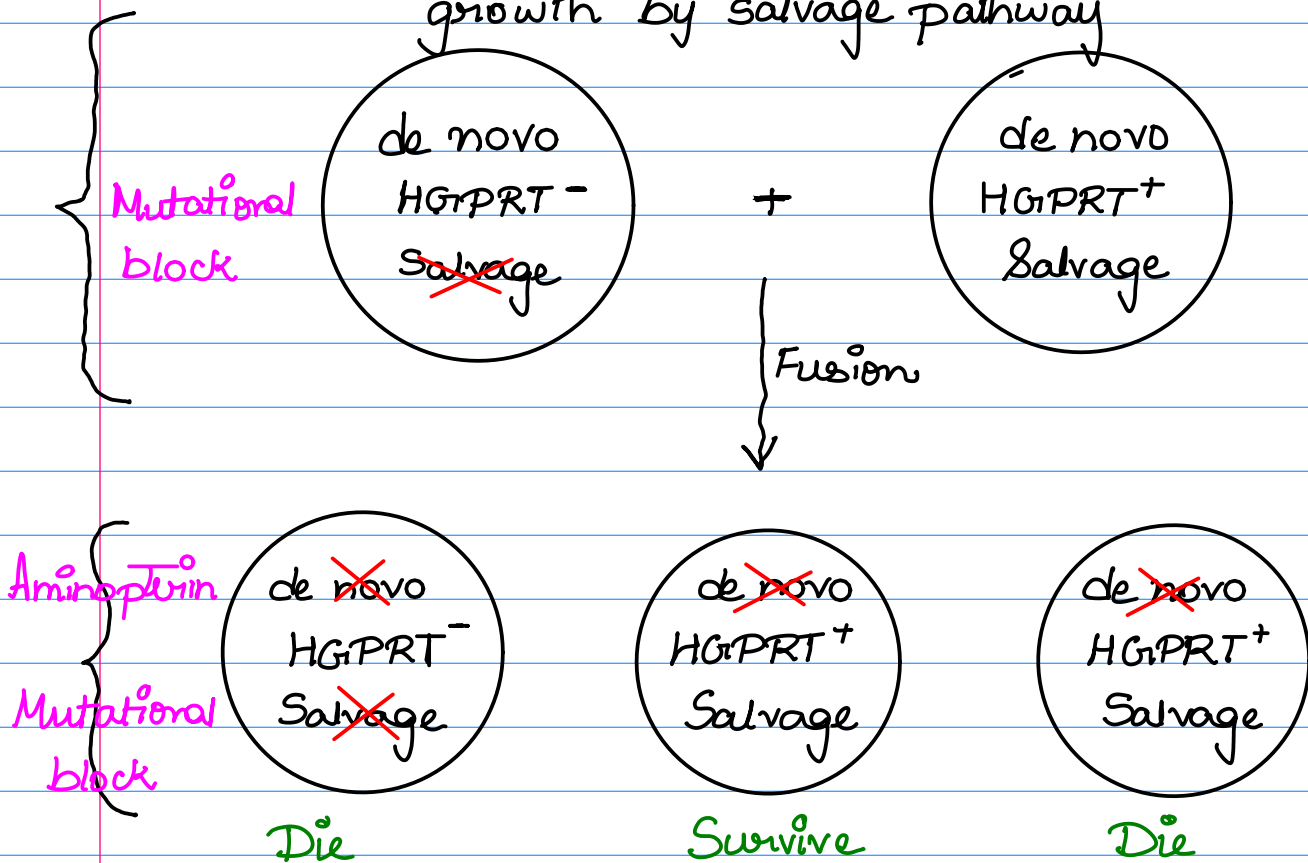


Monoclonal antibodies harvested



HAT Selection

HAT medium \rightarrow **Aminopterin** to block the de novo pathway and hypoxanthine and thymidine to allow growth by salvage pathway



Screening and production of monoclonal antibodies

Culture of hybridoma cells in HAT media



Each well tested for specific antibodies



Screening of hybridoma cells



Expansion of positive clones

Antigen

- antigen – organism, molecule or part of molecule that is recognised by immune system
may be simple, complex, protein, carbohydrate, synthetic in origin
- recognised by immunoglobulin receptor of B cells or by the T cell receptor complexed with MHC

Epitopes

- antigen receptors recognise discrete regions of molecules called **epitopes**
- different lymphocytes, each with a unique set of receptors, may recognise different epitopes on the same antigen

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules