

Monoclonal Antibodies

- Requires cultured myeloma cell lines that grow in normal culture medium but will not grow in a selective media because they lack genes (mutated) required for DNA synthesis. By fusing normal cells to these mutated tumor cells the necessary genes are supplied by the normal cell (B cells) and allow the “hybrid” to grow in the selective media.
- Genes from myeloma cell make “normal B cell” immortal
 - Normal cells synthesize purine nucleotides and thymidylate by a **de novo** pathway requiring tetrahydrofolate
 - Aminopterin-treated cells (inhibitor of folate enzymes) use a **salvage pathway** to make purines and thymidine kinase (TK) makes thymidylate

- HAT media contains
 - **Hypoxanthine:** mutated myeloma cells cannot use hypoxanthine to make purines in the salvage pathway because they are mutated in the HGPRT gene (only mutated tumor cells)
 - **Aminopterin:** stops the action of tetrahydrofolate and cells cannot make purines (both myeloma and normal B cells)
 - **Thymidine:** supplies nucleotide base in media (for both myeloma and normal B cell)
- Unfused myeloma cells will die because they do not have the gene products to replicate DNA– normal B cells will die because they are not immortal– only fused cells will live in HAT medium (immortal genes from myeloma and salvage pathway genes from normal B cell)

