- It is technique of producing hybrid cell lines called "**hybridomas**" by the fusion of fusing a specific **antibody-producing lymphocyte B cell** with a **myeloma cell** that has an ability to grow in tissue culture
- Hydridoma produce antibodies produced that have single specificity and are called **monoclonal antibodies**.
- This technique was discovered Georges Kohler of West Germany, Cesal Milstein of Argentina and Niels Jeme of Denmark in 1975.
- They were awarded Nobel Prize for Physiology and Medicine the 1984

Antibody producing lymphocyte B cell are isolated from the spleen cell of mouse immunized with red blood cells from sheep

Single myeloma cell is a bone marrow tumour cell capable of multiplying indefinitely.

This Cell Line Is Deficient In HGPRT, TK

Fused hybrid cells or hybridoma have the antibody producing capability inherited from lymphocytes using PEG and have the ability to grow continuously or immortal like malignant cancer cells

1. Immunization of a mouse
2. Isolation of B cells from the spleen
3. Cultivation of myeloma cells
4. Fusion of myeloma and B cells
5. Separation of cell lines
6. Screening of suitable cell lines
7. *in vitro* (a) or *in vivo* (b) multiplication
8. Harvesting

Steps in monoclonal antibody production by hybridoma technology

1. Immunize a rabbit through repeated injection of a specific antigen for the production of specific antibody, facilitated due to proliferation of the desired B cells.
2. Produce tumors in a mouse or a rabbit.
3. Culture separately the spleen cells that produce specific antibodies and the myeloma cells that produce tumors
4. Myeloma cells cannot synthesize antibodies as they lack HGPRT gene required for the synthesize the enzyme,

- Fusion of spleen cells to myeloma cells is induced using polyethylene glycol (PEG), to produce hybridoma
- Hybridomas are grown in selective hypoxanthine aminopterin thymidine (HAT) medium.
- HAT medium contains a drug, aminopterin that blocks one pathway for nucleotide synthesis, making the cells dependent on another pathway that needs HGPRT enzyme, which is absent in myeloma cells.

- Prepare single cell colonies that can grow and use them to screen of antibody producing hybridomas
- Only one in several hundred cell hybrids will produce antibodies
- Culture selected hybridoma cells for the production of monoclonal antibodies in large quantities
- Hybridoma cells can be frozen for future use.

Applications

Serological:

- Identification of ABO blood groups.

Diagnosis:

- Detection of pregnancy by assaying of hormones with monoclonals,
- Detection of pathogens
- Separation of one substance from a mixture of very similar molecules.

Immunopurification:

- Purification of individual interferons using monoclonals
- Inactivation of T lymphocytes responsible for rejection of organ transplants.

Therapy:

- Removal of tumour cells from bone marrow.
- Neutralize the reaction or response by one defined antigen,
- treatment of acute renal allograft rejections.
- Treatment -malignant leukemic cells, B cell lymphomas, and a variety of allograft rejections after transplantation.