LEARNING OBJECTIVES

By the end of this presentation, students will be able to:

1. Understand the significance and types of protein drugs in pharmaceutical applications.
2. Explain the concept, development, and applications of monoclonal antibodies (mAbs).
3. Identify key challenges in the production and delivery of protein-based drugs.

DR ALA ABUHAMMAD, PHD

INTRODUCTION TO PROTEIN DRUGS

- Protein drugs are biologically-derived therapeutic molecules, such as hormones, enzymes, or monoclonal antibodies, designed to interact with specific targets in the body.
- Unlike small-molecule drugs, which are chemically synthesized and often have broad mechanisms of action, protein drugs are engineered to work with precision, mimicking natural biological processes.
- Comparison with Small-Molecule Drugs:
  - Protein drugs are larger, more complex, and biologically active compared to chemically synthesized small-molecule drugs.
  - Protein drugs have transformed the management of chronic and life-threatening diseases, offering innovative solutions for conditions that were previously untreatable or poorly managed.
  - at the forefront of personalized medicine, as their development often considers genetic, molecular, or cellular variations in patients.

DIFFERENT TYPES OF PROTEIN THERAPEUTICS

البروتينات إلها أنواع كتير ووظائف مختلفة

Type	Description	Examples	Biotechnological Technique / Approach
Enzymes	Catalyze chemical reactions to replace or augment deficient or abnormal enzymes in patients.	Alteplase (stroke), Pegaspargase (leukemia)	Recombinant DNA technology using bacterial or mammalian cell expression systems.
Monoclonal Antibodies (mAbs)	Target specific antigens, widely used in oncology, autoimmune diseases, and infections.	Rituximab, Trastuzumab, Adalimumab	Hybridoma technology, recombinant DNA technology in mammalian cells (e.g., CHO cells).
Recombinant Hormones	Replace or supplement naturally occurring hormones in cases of deficiency.	Insulin, Growth Hormone, Erythropoietin	Recombinant DNA technology in bacterial (e.g., E. coli) or mammalian cell lines.
Cytokines	Modulate immune responses and stimulate or suppress cellular activity.	Interferons, Interleukins	Recombinant DNA technology in mammalian cell cultures.
Coagulation Factors	Address bleeding disorders by replacing missing or defective clotting proteins.	Factor VIII (Hemophilia A), Factor IX	Recombinant DNA technology in mammalian cells (e.g., CHO or BHK cells).
Fusion Proteins	Combine the functions of different proteins to improve stability or extend half-life.	Etanercept (autoimmune diseases)	Recombinant DNA technology in mammalian cell lines.
Vaccines	Protein-based immunogens that stimulate immune responses for disease prevention.	HPV vaccine, Hepatitis B vaccine	Recombinant DNA technology in yeast (e.g., Saccharomyces cerevisiae) or mammalian cells.
Peptides	Short chains of amino acids used for signaling or therapeutic effects.	Glucagon-like peptide-1 (GLP-1) analogs	Solid-phase peptide synthesis (SPPS) or recombinant technology in bacterial systems.

biotech products بما أنهم يتم تصنيعهم باستخدام biotech technology و تقنيات جديدة زاي recombinant DNA و أضدادها مثل hybridoma

ADVANTAGES OF PROTEIN DRUGS

(قرأته قراءة)

- **Precision:**
  Protein drugs exhibit highly specific interactions with their targets, which is a key advantage over many small-molecule drugs. These precise interactions arise from the ability of proteins to recognize and bind to unique molecular structures on target cells or pathways. This specificity significantly reduces the likelihood of off-target effects, thereby minimizing adverse reactions and increasing the drug's safety profile.
- **Safety:** ← وهاد بنعكس على non selective ممكن تكون precision عندهم موديماً يكون
  Protein drugs are generally better tolerated by patients because they often mimic the body's natural biological molecules and processes. Unlike some small-molecule drugs that may cause toxicity or disrupt unrelated pathways, protein drugs integrate more seamlessly with biological systems, leading to fewer and less severe side effects.
- **Therapeutic Versatility:**
  Protein drugs can perform a wide range of therapeutic roles. They can:
- Replace deficient or malfunctioning proteins in the body, such as insulin for diabetes.
- Act as modulators of immune responses, helping to enhance or suppress the immune system in conditions like autoimmune diseases or cancer.
- Serve as signaling molecules that regulate specific physiological processes, such as growth factors stimulating tissue regeneration or repair.
- **Efficacy:**
  Protein drugs often surpass traditional therapies in effectiveness because they can closely mimic natural biological functions. For instance, monoclonal antibodies can specifically target cancer cells without harming healthy tissues. Their ability to bind strongly and selectively to their targets ensures high therapeutic impact, even at lower dosages compared to small-molecule drugs.

CHALLENGES OF PROTEIN DRUGS

- 1. Stability Issues
- **Sensitivity to Environmental Conditions**: Protein drugs are highly sensitive to changes in temperature, pH, and enzymatic degradation. This makes handling, storage, and transportation difficult, requiring strict cold-chain logistics.
- **Denaturation Risks**: During formulation or exposure to unfavorable conditions, protein drugs may lose their functional structure, reducing efficacy.
- 2. High Manufacturing Costs
- **Complex Production Systems**: The production of protein drugs involves sophisticated bioreactors and advanced technology, significantly driving up costs.
- **Purification Requirements**: Extensive downstream purification processes are essential to achieve high-purity protein drugs, adding to the expense.
- 3. Immunogenicity
- **Immune Reactions**: Protein drugs may be recognized as foreign by the immune system, triggering adverse reactions.
- **Mitigation Strategies**: Developers must employ careful molecular design, including PEGylation or glycosylation, to minimize immunogenic responses.
- 4. Short Shelf Life
- **Degradation Over Time**: Protein drugs often have limited stability over extended periods, leading to reduced therapeutic effectiveness.
- **Stringent Storage Needs**: Specialized storage conditions, such as refrigeration, are mandatory to maintain drug integrity and potency.

PRODUCTION OF PROTEIN DRUGS

- Host Systems:
- Bacteria (e.g., E. coli): Cost-effective but limited post-translational modifications.
- Yeast: Simple eukaryotic system with glycosylation capabilities.
- Mammalian Cells (e.g., CHO cells): Preferred for complex proteins requiring human-like modifications.
- **Recombinant DNA Technology**: Gene encoding the protein of interest is inserted into a vector for expression in the host system.
- **Processes**: Upstream (cell culture) and downstream (purification, formulation).

KEY APPLICATIONS OF PROTEIN DRUGS

(قراءة)

- **Replacement Therapies:**
  - Insulin for diabetes.
  - Growth hormone for GH deficiency.
- **Enzyme Therapies:** E.g., agalsidase beta for Fabry disease.
- **Immunotherapy:** Interferons for viral infections and certain cancers.
- **Targeted Therapy:** E.g., rituximab for B-cell malignancies.

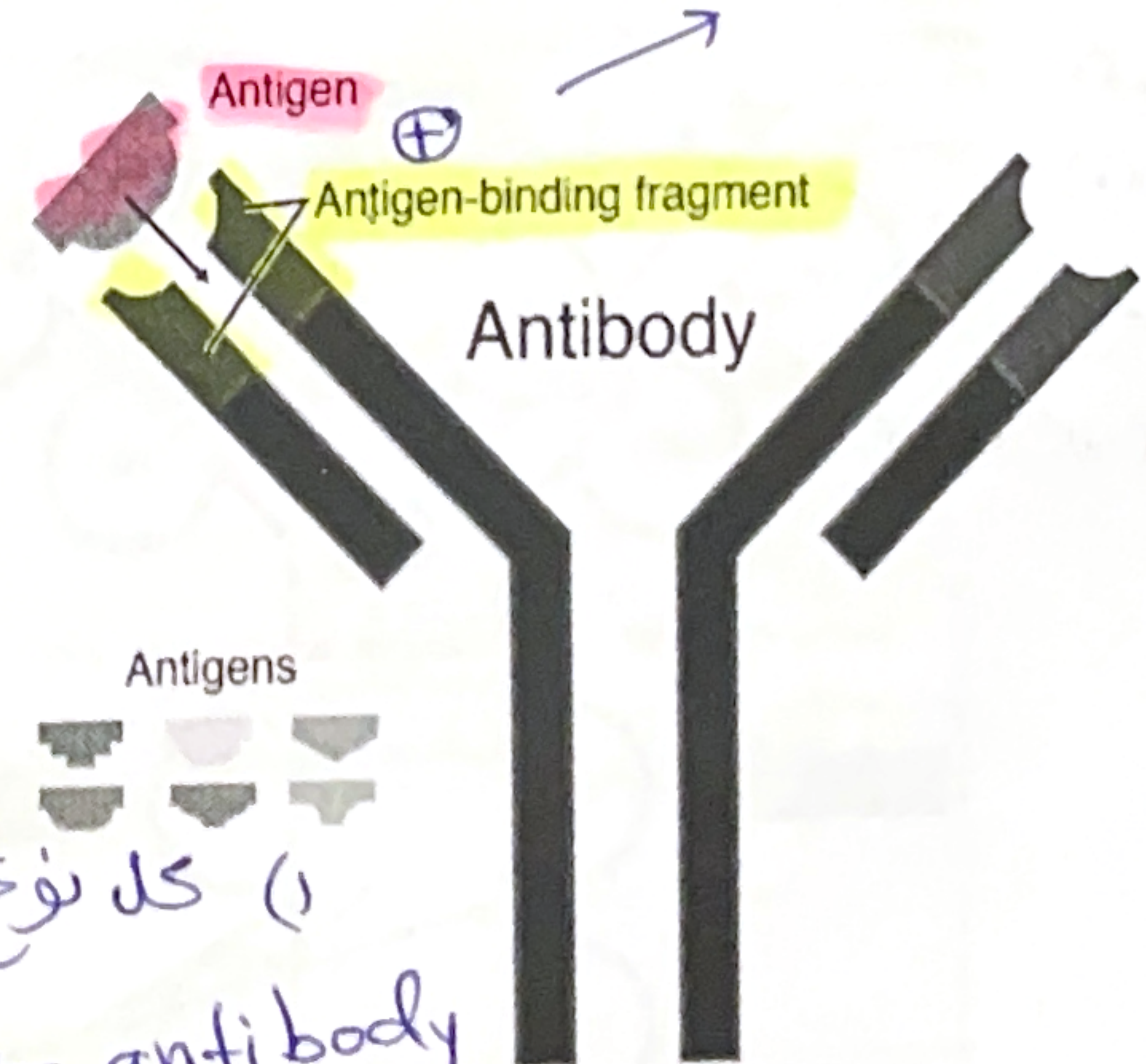
ANTIBODY

بس يرتبطو بصير عنا neutralization ثم بتبعها عملية engulfying by immune cells

1) An antibody is a protein used by immune system to identify and neutralize ***foreign objects*** like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target.

2) The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms.

3) With in vitro assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.



1) كل نوع forign object بتسبب إنتاج نوع معين من antibody ← to neutralize this forign object

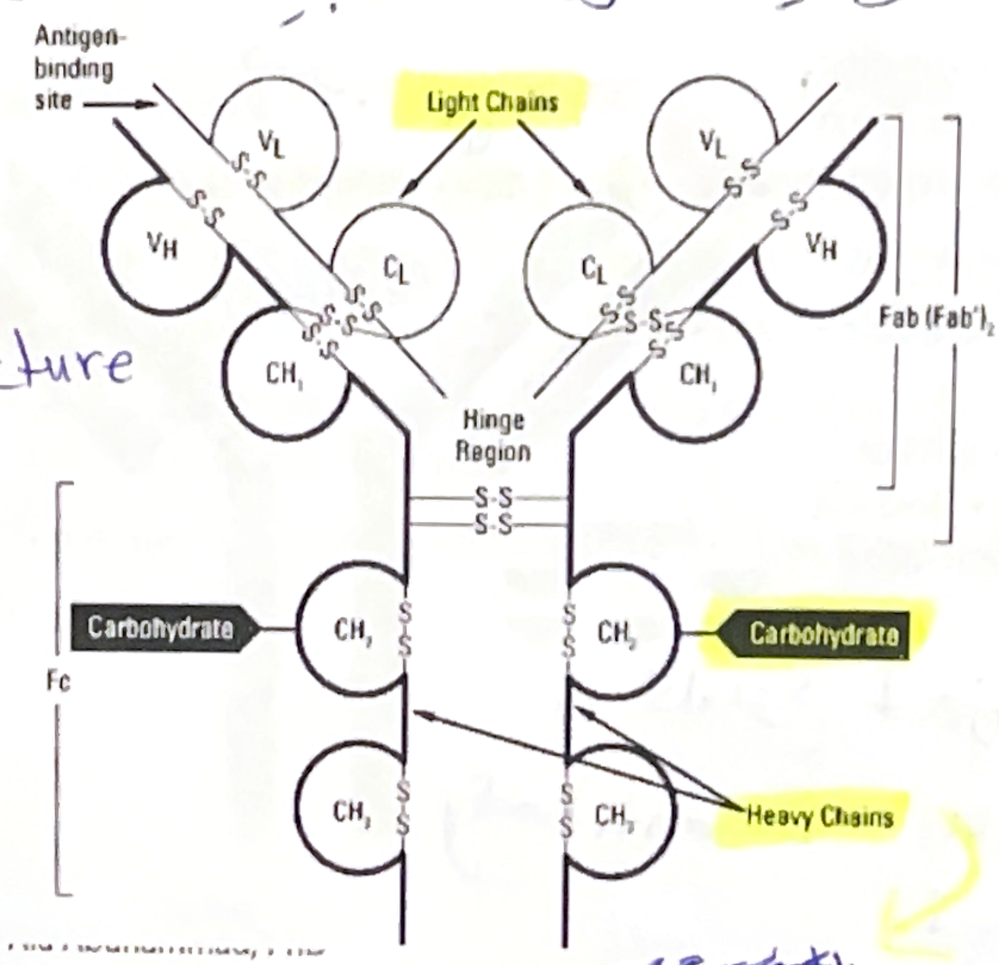
ANTIBODIES (IMMUNOGLOBULINS)

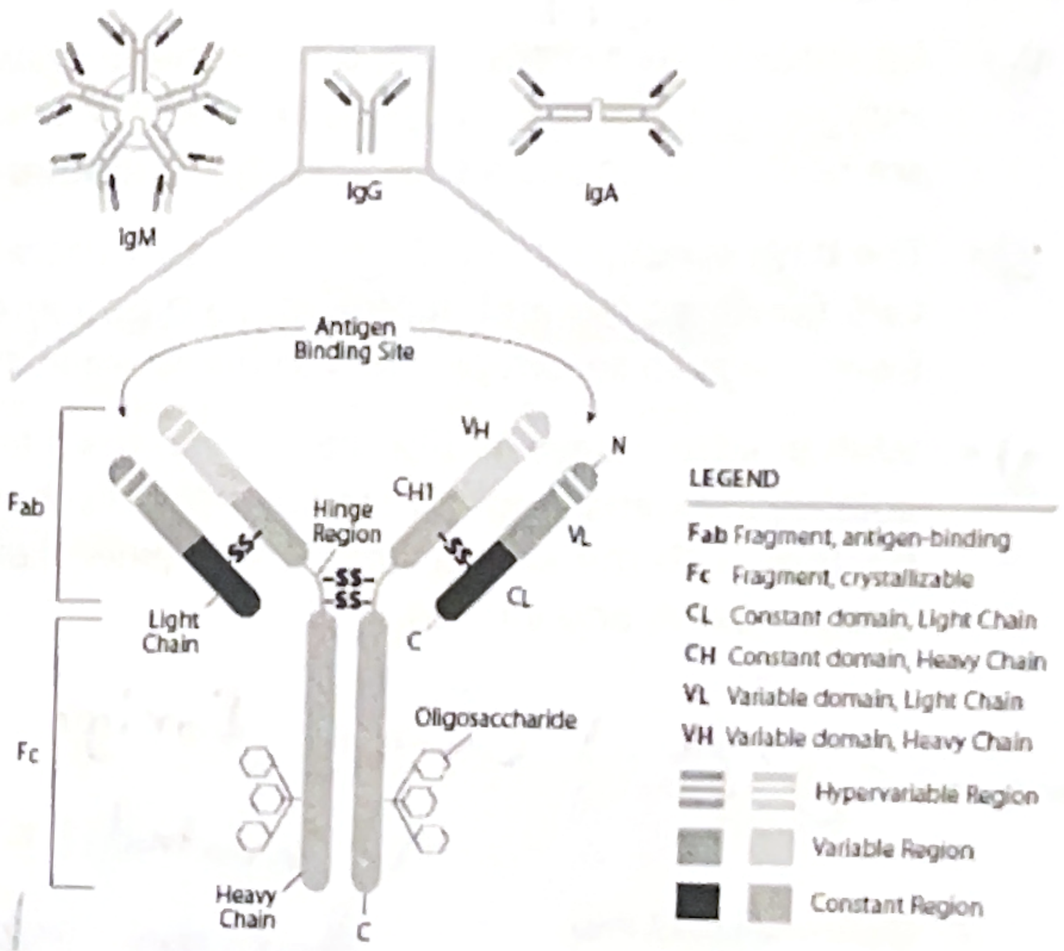
(قراءة)

- Immunoglobulins, also known as antibodies, are **glycoprotein** molecules produced by plasma cells (white blood cells).
- They act as a critical part of the immune response by specifically recognizing and binding to particular antigens, such as bacteria or viruses, and aiding in their destruction. The antibody immune response is highly complex and exceedingly specific.
- The various immunoglobulin classes and subclasses (isotypes) differ in their biological features, structure, target specificity, and distribution. Hence, the assessment of the immunoglobulin isotype can provide useful insight into the complex humoral immune response. Assessment and knowledge of immunoglobulin structure and classes is also important for selection and preparation of antibodies as tools for immunoassays and other detection applications.

IMMUNOGLOBULIN STRUCTURE AND CLASSES

← هاي الصورة بتفرضنا الفروقات بين different classes وبيه هو الفرق بال structure

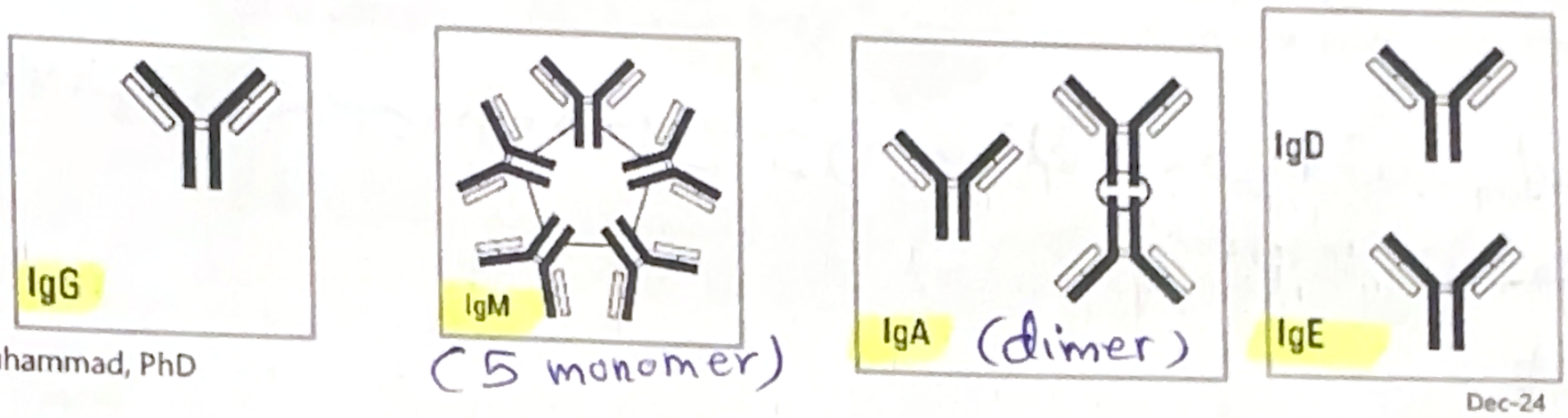




الاختلافات جميعهم هي اللي بتأدي إلى اختلافات بال different classes

CLASSES OF IMMUNOGLOBULINS

- immunoglobulins classes are distinguished by the type of heavy chain found in the molecule.
- Differences in heavy chain polypeptides allow these immunoglobulins to function in different types of immune responses and at particular stages of the immune response.
- Antibody classes differ in valency as a result of different numbers of Y-like units (monomers) that join to form the complete protein. For example, in humans, functioning IgM antibodies have five Y-shaped units (pentamer) containing a total of ten light chains, ten heavy chains, and ten antigen-binding.



(5 monomer)

(dimer)

← الفروقات بينهم بتكون على مستوى monomer نفسه والـ valency يلي راح تأثر على كيف هدول monomers رح يرتبطو مع بعض عشان يعطوني الـ isotype أو subtype النهائي

CLASSES OF IMMUNOGLOBULINS

(قراءة) ، الارقام موحفظ

Important properties of antibody isotypes.

	IgG	IgM	IgA	IgE	IgD
Molecular weight	150,000	900,000	320,000 (secretory)		
Heavy chain: Type MW	γ 53,000	μ 65,000	α 55,000	ε 73,000	δ 70,000
Concentration in serum (approximate)	10-16mg/mL	0.5-2mg/mL	1-4mg/mL	0.00001-0.0004mg/mL	0-0.4mg/mL
Percent of total IgG	80	6	13	0.002	0.2
Carbohydrate (approximate)	3%	12%	10%	12%	13%
Distribution	Intravascular and extravascular	Mostly intravascular	Intravascular and secretions	Basophils and mast cells in saliva and nasal secretions	Lymphocyte surface
Function	Secondary response	Primary response	Protect mucous membranes	Protect against parasites	Unknown
Structure					

جدول حفظ

SUBCLASSES OF IMMUNOGLOBULINS

- In addition to the major immunoglobulin classes, several Ig subclasses exist in all members of a particular animal species. Antibodies are classified into subclasses based on minor differences in the heavy chain type of each Ig class. In humans there are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4 (numbered in order of decreasing concentration in serum).
- Variance among different subclasses is less than the variance among different classes. For example, IgG1 is more closely related to IgG2, IgG3 and IgG4 than to IgA, IgM, IgD, or IgE. Consequently, antibody-binding proteins (e.g., Protein A or Protein G) and most secondary antibodies used in immunodetection methods cross-react with multiple subclasses but usually not multiple classes of Ig.

الفروقات بينهم موجودة بكثرة ، مثلاً لو بدي أقارن أكيد هعمل بشوف بعض أكثر وأقرب

IgG1, IgG2, IgE

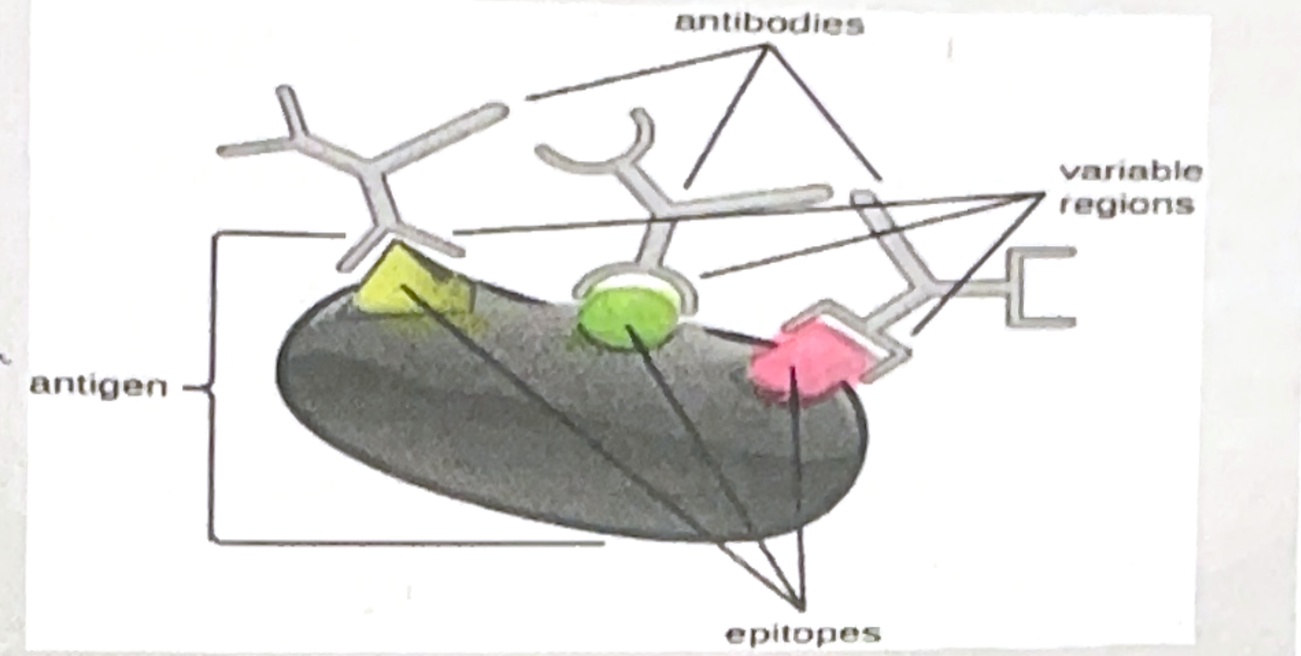
بال structure

therapeutic protein من أشهر وأهم الأمثلة عن (بتم تصنيعهم فارغ اكس AB)

MONOCLONAL ANTIBODIES (mAbs): AN OVERVIEW

- **Definition:** Monoclonal antibodies (mAbs) are lab-engineered antibodies designed to target specific antigens with high precision.
- **Historical Perspective:** Developed using hybridoma technology (Milstein and Köhler, *Nobel Prize*, 1984).
- **Applications:** Used in oncology (e.g., rituximab), autoimmune disorders (e.g., infliximab), and infectious diseases.
- Examples:
  - **Infliximab**: Treats autoimmune diseases (e.g., rheumatoid arthritis, Crohn's disease).
  - **Trastuzumab**: Used for HER2-positive breast cancer.

MONOCLONAL ANTIBODIES (mAbs)



An antibody binds to a specific region on an antigen called an epitope. A single antigen can have multiple epitopes for different, specific antibodies.

MONOCLONAL ANTIBODIES (mAbs)

- Monoclonal antibodies are identical immunoglobulins, generated from a single B-cell clone. These antibodies recognize unique epitopes, or binding sites, on a single antigen. Derivation from a single B-cell clones and subsequent targeting of a single epitope is what differentiates monoclonal antibodies from polyclonal antibodies.

- Polyclonal antibodies are antibodies that are derived from different cell lines. They differ in amino acid sequences.

(قرأته مراده)

POLYCLONAL VS. MONOCLONAL ANTIBODIES

Feature	Polyclonal Antibodies	Monoclonal Antibodies
Definition	Mixture of antibodies recognizing multiple epitopes on the same antigen.	Identical antibodies targeting a single epitope.
Source	Produced by different B cell clones in an immunized animal's serum.	Produced by hybridoma cell lines (B lymphocytes fused with myeloma cells).
Heterogeneity	Heterogeneous (varied antigen-binding specificities).	Homogeneous (single specificity).
Applications	Secondary antibodies in immunoassays.	Primary antibodies for single-epitope specificity.
Advantages	- Broad recognition of epitopes. - Cost-effective.	- High specificity. - Consistent supply.
Production	Directly purified from serum.	Grown in cell culture or collected from ascites fluid.

بما انها selective أكثر تصنيعهم متطلب أكثر
وايضاً مكلف أكثر

ANTIBODY PRODUCTION

- Antibody production involves preparation of antigen samples and their safe injection into laboratory or farm animals so as to evoke high expression levels of antigen-specific antibodies in the serum, which can then be recovered from the animal.
- Polyclonal antibodies are recovered directly from serum (bleeds).
- Monoclonal antibodies are produced by fusing antibody-secreting spleen cells from immunized mice with immortal myeloma cell to create monoclonal **hybridoma** cell lines that express the specific antibody in cell culture supernatant.

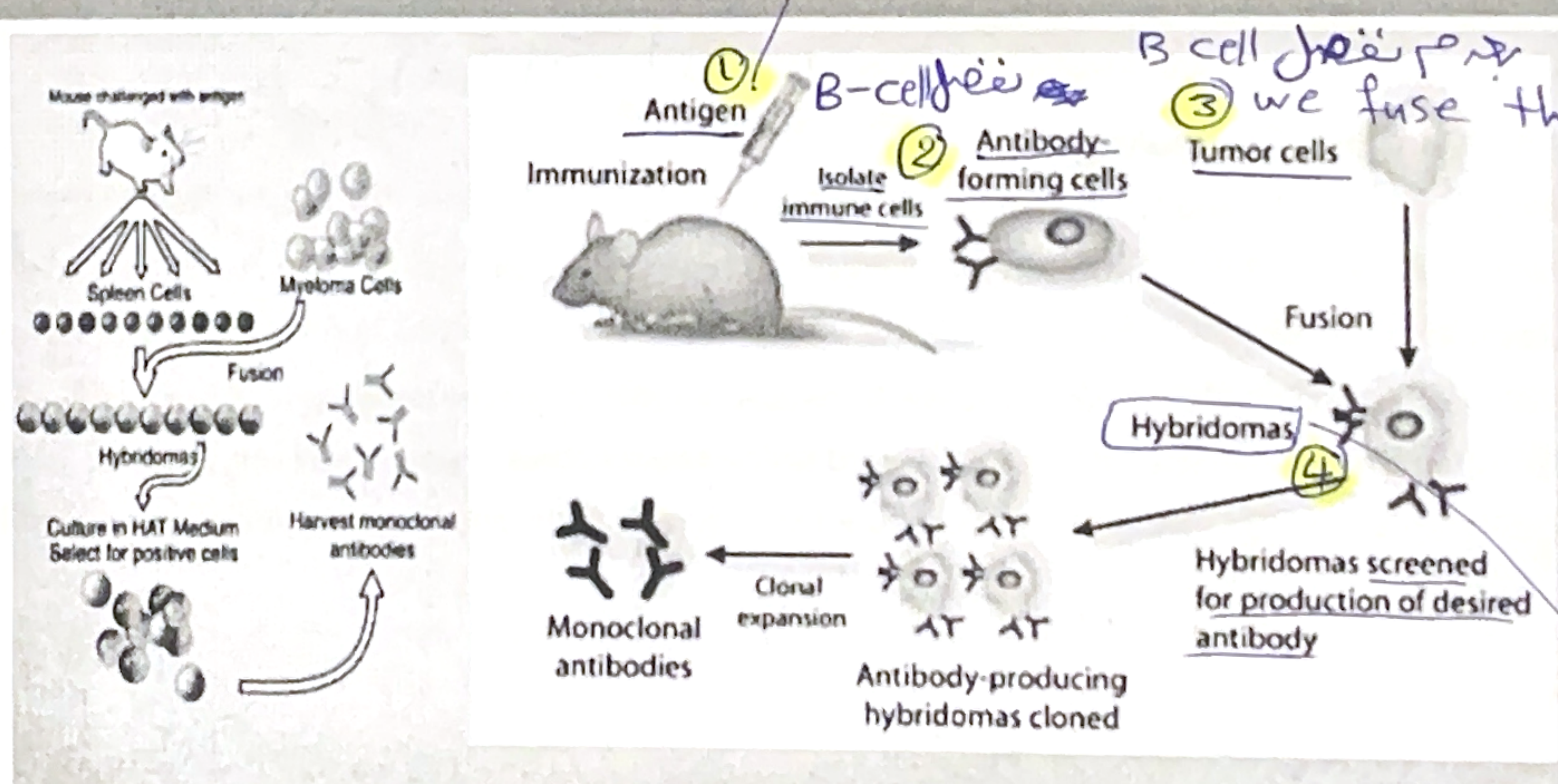
PRACTICAL STEPS FOR mAbs PRODUCTION

- 1. Immunize animal
- 2. Isolate spleen cells (containing antibody-producing B cell)
- 3. Fuse spleen cells with myeloma cell (using PEG)
- 4. Allow unfused B cell to die
- 5. Add aminopterin to culture and kill unfused myeloma cells
- 5. Clone remaining cells (place 1 cell/well and allow each cell to grow into a clones of cell)
- 7. Screen supernatant of each clone for presence of desired antibody
- 8. Grow chosen clone of cells in tissue culture indefinitely
- 9. Harvest antibody from the culture.
- 10. $1000-2000 per mg

⑤ بما انه حطيت بال culture cell ← (hybridoma ← tumor + B cell)
راح يكون عنا mixture منهم الثلاث، كيف بدي أعمل select فقط للhybridoma وأتخلص منالباقي؟ بحطهم في certain media (HAT) ← وجود هاي media بسمح فقط للhybridoma cells انها survive وال B-cell, tumor cells ← بتموت بهاي media

PRACTICAL STEPS FOR PRODUCTION

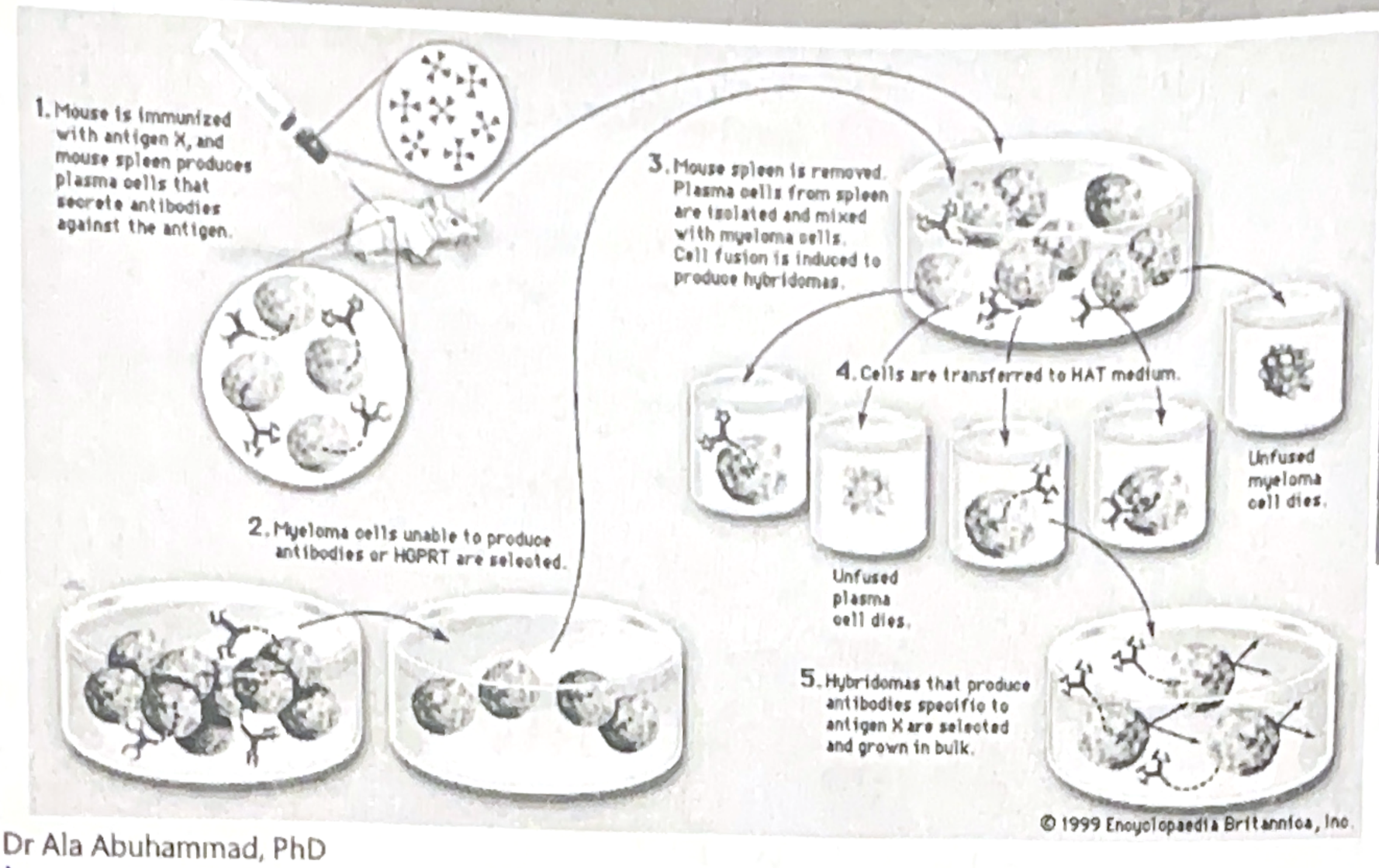
introduce a trigger (antigen) to the animal
عشان يخلي الحيوان يصنع AB



Preparation of Monoclonal Antibodies

هاي الخلايا تتكون أخذت خصائص cancer cells (صار عمرهم أطول وبتكاثرو أسرع) وأخذت خصائص B-cell (قادرين يصنعوا antibody of interest)

PRACTICAL STEPS FOR PRODUCTION



Read more: why hyprodoma cells can survive the HAT medium?

← الصورة هاي فيها تفاصيل أكثر (ما شرحتها)

وبتحكيلكم الدكتورة أقرأ أو أكثر عن HAT media

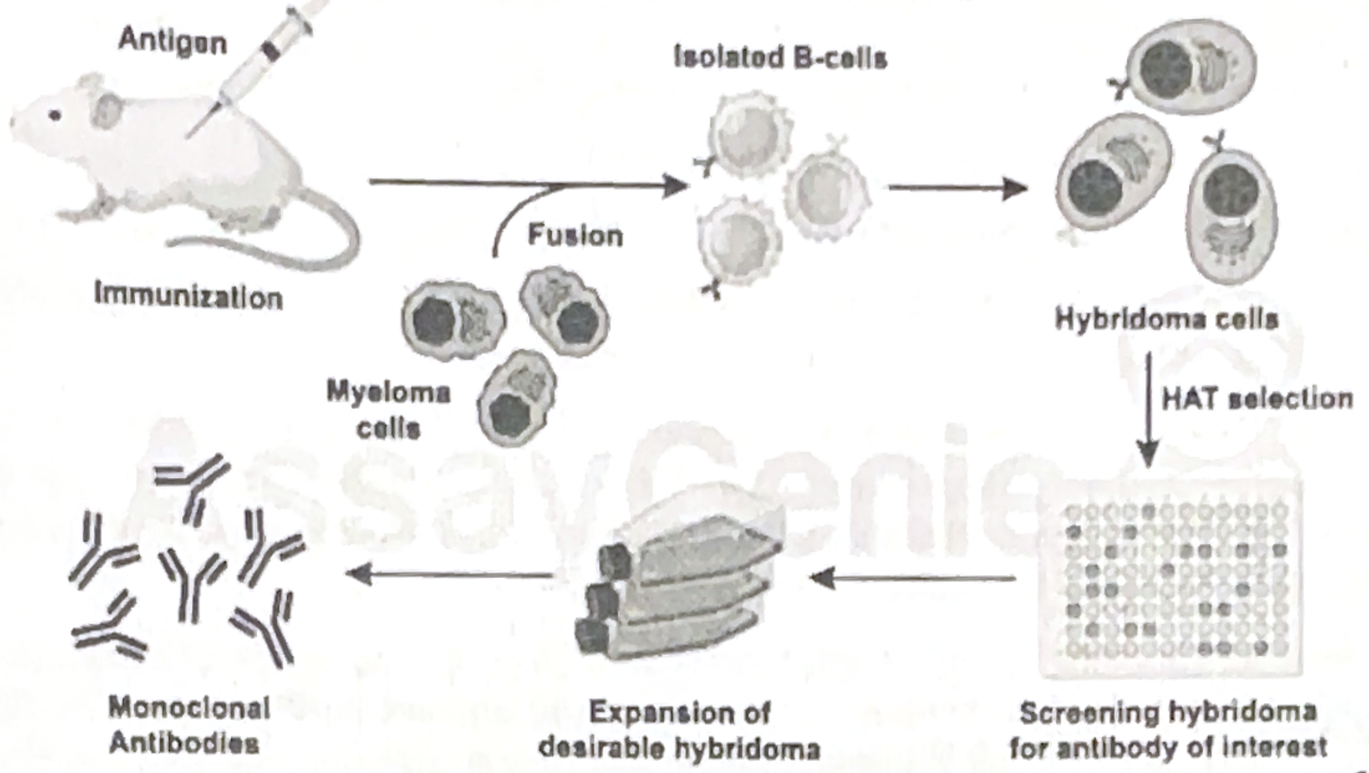
Hybridoma Technology: Revolutionizing Antibody Production!

(قرأتها قراءة)

- What is Hybridoma Technology?
  - Developed by Köhler and Milstein in the 1970s (*Nobel prize*).
  - Fusion of B cells (antibody-producing) and myeloma cells (immortal).
- Key Steps in the Process:
  - **Immunization:** Small mammal (e.g., mouse) is immunized with a specific antigen.
  - **Cell Fusion:** B cells and myeloma cells are fused to form hybridomas.
  - **Selection & Screening:** Hybridomas are cultured in selective media, and those producing desired antibodies are isolated.
  - **Cloning:** Ensures consistent and limitless monoclonal antibody supply.
- Significance:
  - Provides antibodies with identical specificity.
  - Applications in **diagnostics**, **therapeutics**, and **research.**

HYBRIDOMA TECHNOLOGY

HYBRIDOMA TECHNOLOGY



ANTIBODY PURIFICATION

- Antibody purification involves isolation of antibody from serum (polyclonal antibody), ascites fluid or culture supernatant of a hybridoma cell line (monoclonal antibody).
- Purification methods range from very crude to highly specific:
  - **Crude**—precipitation of a subset of total serum proteins that includes immunoglobulins
  - **General**—affinity purification of certain antibody classes (e.g., IgG) without regard to antigen specificity
  - **Specific**—affinity purification of only those antibodies in a sample that bind to a particular antigen molecule
- Which level of purification is necessary to obtain usable antibody depends upon the intended application(s) for the antibody.

← عشان تصنّع اي دوا أو اي منتج لازم يكون عندك tools الجهة يلي تمكنك انك تعمل quality control and quality assurance production of the product

ANTIBODY CHARACTERIZATION

mono بالنسبة للـ AB عنا هدول

- Antibody characterization involves three kinds of activities that are usually performed at various stages throughout an entire antibody production and purification project:
  1. Screening—identifying antibody samples having antigen-binding specificity
  2. Titering—measuring antibody concentration and functional assay titer
  3. Isotyping—determining a monoclonal antibody class and subclass identity
- **Screening** is first required during production to identify which animals and hybridoma clones are producing a high level of antigen-specific antibody. This is usually accomplished using ELISA techniques.
- **Antibody concentration** can be estimated using either a general protein assay or a species- and immunoglobulin-specific method, such as with specialized microagglutination assay kits. Antibody titer is related to concentration but refers more specifically to the effective potency of a given antibody sample. Measuring titer usually means determining the functional dilution of an antibody sample necessary for detection in a given assay, such as ELISA.
- **Isotyping** involves determining the class (e.g., IgG vs. IgM) and subclass (e.g., IgG1 vs. IgG2a) of a monoclonal antibody. This is a critical step in antibody production, as it is necessary for choosing an appropriate purification and modification method for the molecule. Isotyping is most easily accomplished with commercial, ready-to-use antibody isotyping kits.

screening ← مثلاً كنت عم تعمل immunization لأكثر من mouse مثلاً (5)، لازم يكون عندك طريقة واضحة بنهاية التجربة عشان تعمل screening وتاخذ mouse يلي عمل انتاج للـ AB يلي بدك اياه

ANTIBODY FRAGMENTATION

- Purified antibodies can be modified for particular uses by several methods including fragmentation into smaller antigen-binding units, conjugation with enzyme or other detectable markers, and immobilization to solid supports.
- Most often antibodies are used in whole-molecule form. However, the performance of some techniques and experiments can be improved by using antibodies whose nonessential portions have been removed.
- Antibody fragmentation refers to procedures for cleaving apart whole antibody molecules and removing portions that are not necessary for binding antigen. Fab and F(ab)'2 are antibody fragments of IgG that are most frequently created and utilized by researchers.

← f أحياناً بعض application ما بكون بهمني أو بعنيني اني استخدم whole AB يلي عنا، فبعملو fragment للـ AB عشان آخذ الـ part المهم لهاد application

ANTIBODY LABELING AND IMMOBILIZATION

- Antibodies are produced and purified for use as antigen-specific probes. However, their utility in any given technique (ELISA, western blotting, cellular imaging, immunohistochemistry) depends upon having a mechanism to secondarily detect the antibody.
- Techniques that utilize antibodies for immunoprecipitation or other form of affinity purification depend upon mechanisms for attaching or immobilizing them to chromatography media (e.g., beaded agarose resin). Strategies for accomplishing this involve the same considerations and chemical methods as antibody labeling.

← في بعض أنواع assay برضو بنحتاج نعمل labelling أو (labelling using radio active or flourcencent) حتى surface عن antigen الـ immobilization أو يكون immobilize يعني مثبتين (fixed) على سطح معين ويكون بـ application مثل elisa

Applications Of Antibodies: Diagnostic, Therapeutic, And Research Tools (ماشرحتها)

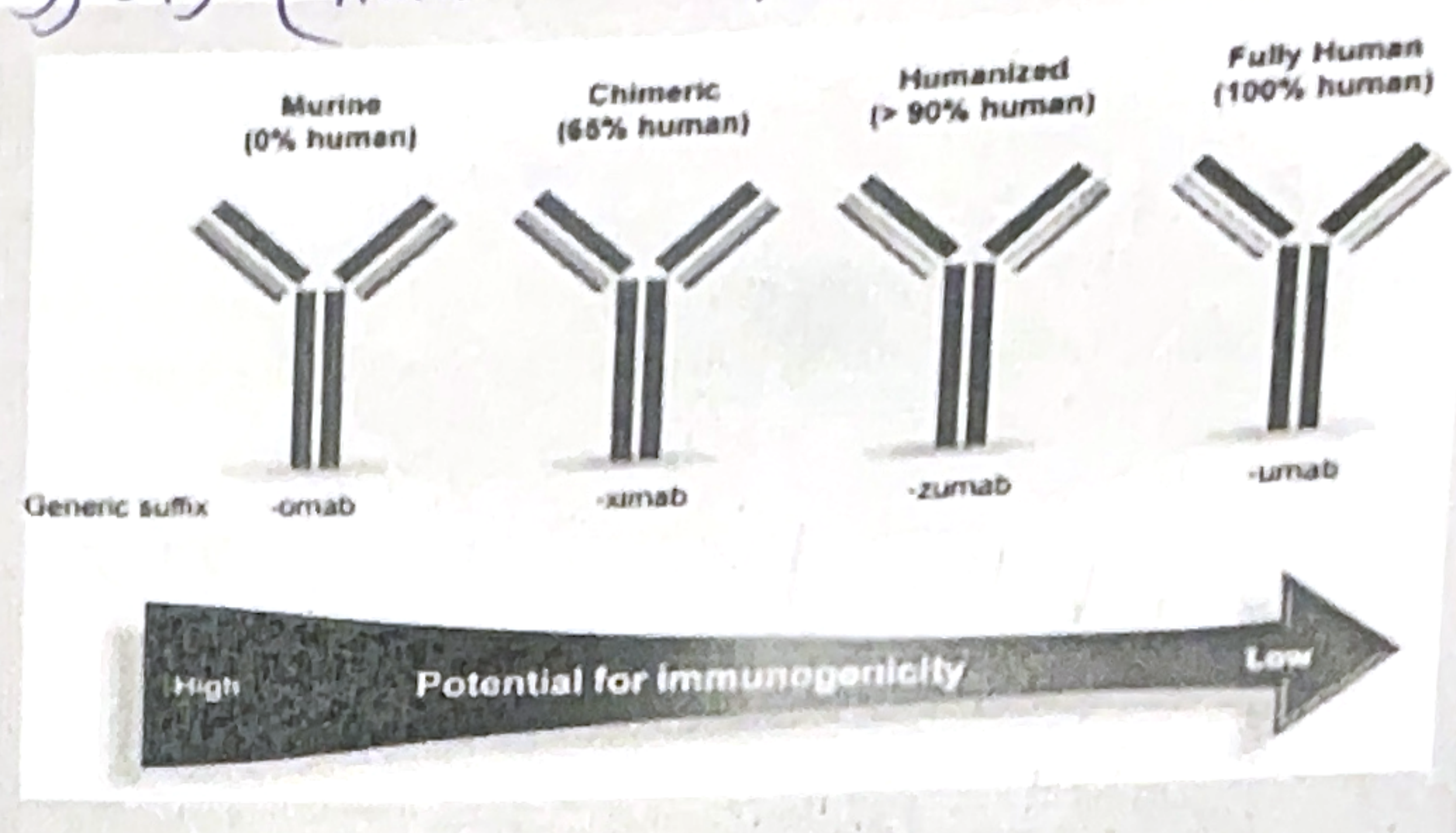
- Antibodies are indispensable tools in both research and clinical settings, owing to their ability to specifically bind to target antigens. Their applications include:
  - **Diagnostic Tools**: Antibodies are fundamental in assays such as ELISA and Western blotting, enabling the detection and quantification of specific proteins or antigens.
  - **Therapeutic Agents**: Monoclonal antibodies are employed in treating various diseases, including cancers and autoimmune disorders, by targeting specific cellular antigens.
  - **Research Applications**: In molecular and cellular biology, antibodies facilitate the study of protein expression, localization, and function through techniques like immunofluorescence and flow cytometry.
  - **Custom Antibody Development**: Tailored antibodies are generated to meet specific research needs, ensuring optimal performance in designated applications.

https://www.thermofisher.com/jo/en/home/life-science/antibodies.html

TYPES OF MONOCLONAL ANTIBODIES

ممكن نقسمها لأنواع حسب المصدر لل AB
(وقديه أقرب لل human) (الفروقات بتيجي من host إلي استخدمته لتصنيع AB)

1. **Murine:** Fully mouse-derived, leading to high immunogenicity (e.g., muromonab-CD3) - limited
2. **Chimeric:** Partly human (70%), reduced immunogenicity (e.g., infliximab).
3. **Humanized:** ~90% human; engineered to retain antigen-binding specificity (e.g., trastuzumab).
4. **Fully Human:** Developed using transgenic mice (1) or phage display (2), minimizing immune responses (e.g., adalimumab).



Immunogenicity potential based on source of Ig

بجيبو mice وبعملو عليه genetic modefication
بشيلو الجين المسؤول عن تصنيع AB من mouse وبحطو محلو الجين المسؤول عن تصنيع AB من human

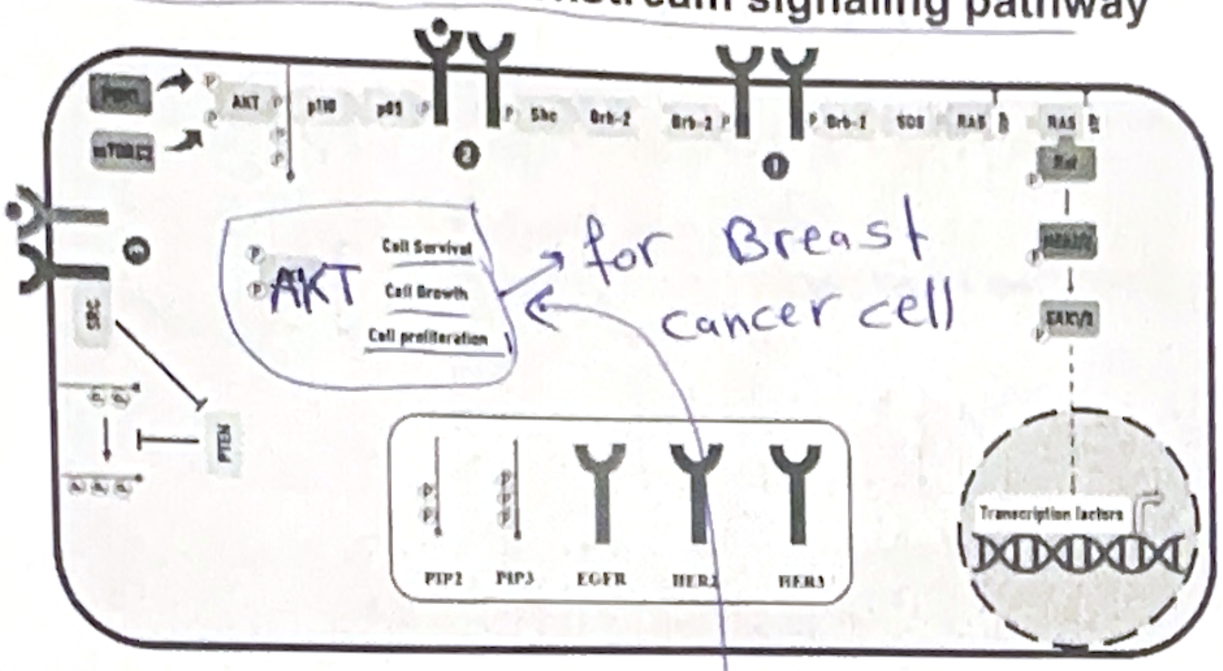
MONOCLONAL ANTIBODY PRODUCTION

- **Antigen Selection:** Identify and purify the target antigen.
- **Hybridoma Technology:** Fuse antibody-producing B-cells with immortal myeloma cells to create hybridomas.
- **Screening:** Select hybridomas producing the desired antibody.
- **Purification:** Chromatographic techniques ensure high-purity mAbs.

APPLICATIONS OF MONOCLONAL ANTIBODIES

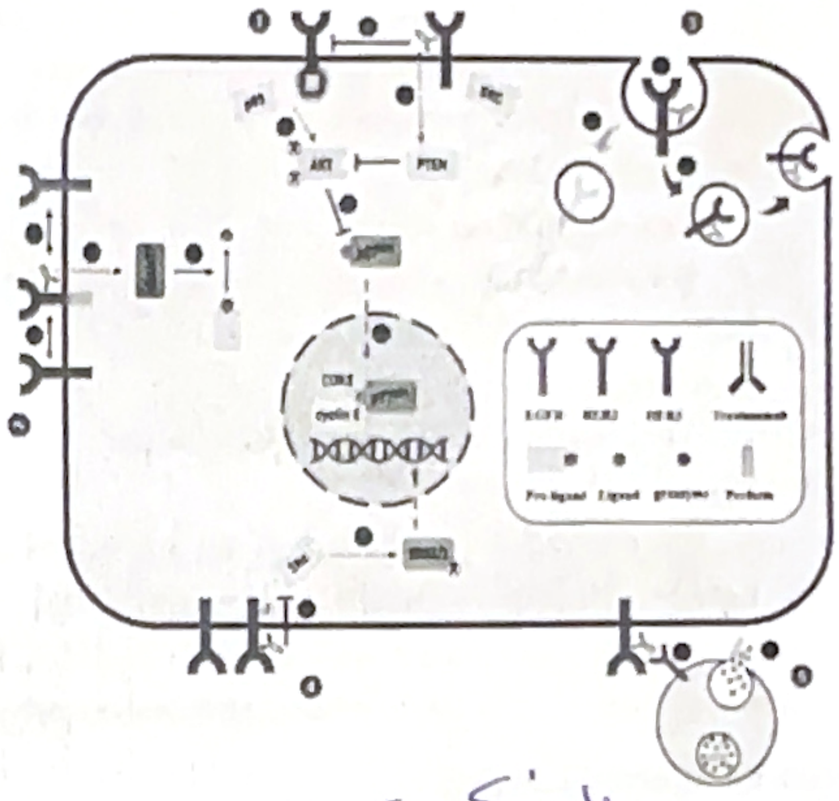
- Cancer Therapy:
  - HER2-positive breast cancer (trastuzumab). → block Her 2 recepter

HER2-mediated downstream signaling pathway



for Breast cancer cell

Trastuzumab mechanisms of action



Dr Ala Abuhammad, PhD

long signaling Cascade بكون مرتبط Her 2 الفكرة: داخل الخلية، فلما أعمل تثبيط لها signaling Cascade، Her2 كله راح يتأثر وبالنهاية على

CASE STUDY: TRASTUZUMAB

- **Target:** HER2 receptor in breast cancer.
- **Mechanism of Action:**
  - Blocks HER2 signaling.
  - Mediates antibody-dependent cellular cytotoxicity.
- **Challenges:**
  - Resistance development. → trastuzumb لا يناسب كل أنواع أدوية الـ Cancer
  - Strategies: Combination with pertuzumab and chemotherapy. (chemoagent → small molecules)

لمهاجم الخلايا السرطانية مباشرة مما يجعل الأورام أقل قدرة على التطور ومقاومة

Mechanisms Of Action Of Trastuzumab

The Hashemite University

- Inhibition of HER2 Signaling:
  - Blocks ligand-independent **HER2-HER3 heterodimer formation** and HER3 phosphorylation.
  - Prevents **PI3K-AKT signaling** by:
    - Inhibiting PI3K complex formation.
    - Activating **PTEN phosphatase**, reducing AKT phosphorylation.
  - Reduces cell proliferation via **p27KIP1 activation**, which:
    - Enters the nucleus and suppresses CDK2/cyclin E activity, arresting the cell cycle.
- Increased HER2 Activity:
  - Promotes **HER2 homodimer formation**.
  - Enhances **ADAM17 expression**, increasing HER-specific ligand production and **HER2 heterodimer formation**.
- HER2 Internalization and Fate:
  - Induces HER2 **internalization**, leading to:
    - **Degradation** or **recycling** to the cell membrane.
- Inhibition of ERK Pathway:
  - Dissociates **Shc from HER2 homodimers**, reducing **ERK1/2 phosphorylation** (via unknown mechanisms).
- Antibody-Dependent Cellular Cytotoxicity (ADCC):
  - Fc region binds to FcγRs on immune effector cells (e.g., NK cells).
  - Activates immune cells to release **perforin and granzymes**, inducing tumor cell apoptosis.

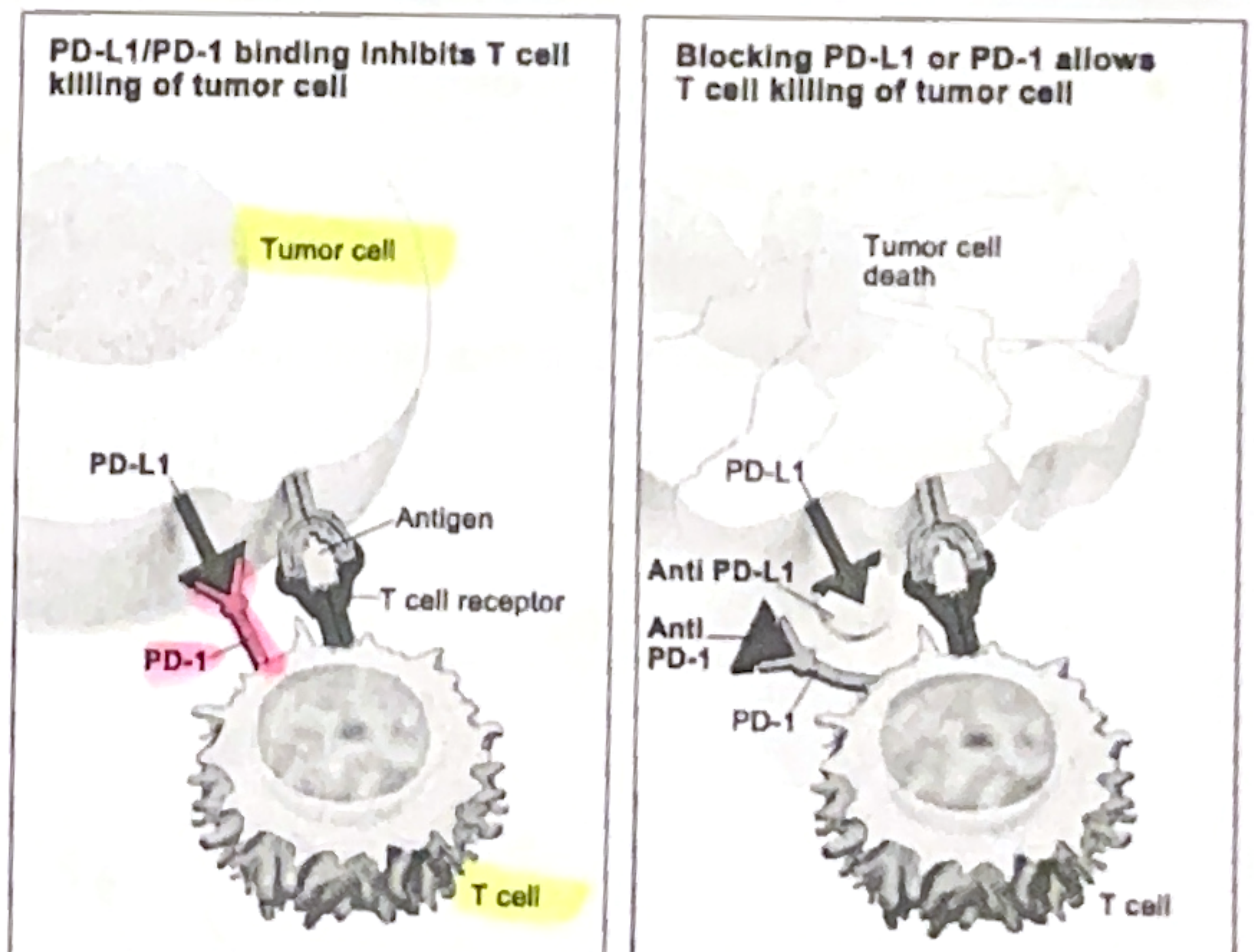
ما شرحتها هاي السلايد (بتحكيلكم اقرأوها)

تابع ① ممكن باستخدام immuno therapy أصنع نوع معين من antibody ترتبط بال receptors المسؤولة عن immune check point فتمنع T cell انها تتعرف على الخلية في جسم الانسان انها خلية عادية بالتالي تؤدي إلى التهامها (Killing of tumor cell using immune cells)

APPLICATIONS OF MONOCLONAL ANTIBODIES

The Hashemite University

- Cancer Therapy:
  - Immune checkpoint inhibitors (e.g., pembrolizumab).
    - Immunotherapy drugs called immune checkpoint inhibitors work by blocking checkpoint proteins from binding with their partner proteins. This prevents the "off" signal from being sent, allowing the T cells to kill cancer cells.
    - Immune checkpoints are a normal part of the immune system. Their role is to prevent an immune response from being so strong that it destroys healthy cells in the body.
    - The US FDA has successfully approved three different categories of immune checkpoint inhibitors (ICIs) such as PD-1 inhibitors (Nivolumab, Pembrolizumab, and Cemiplimab), PDL-1 inhibitors (Atezolimumab, Durvalumab and Avelumab), and CTLA-4 inhibitor (Ipilimumab).



① فكرتهم بشكل عام انو هدول mono AB بقدرو يرتبطو بإشارة immune check point (هي اماكن بتشيك من خلالها الخلية [تحديداً T cell] ازا ارتبطت على خلية ثانية زي tumor cell ← it will not identified it as a forien cell

أماكن معينة

(توضيح) ← (هي عبارة عن بروتينات موجودة في

* immune checkpoints ← هي آليات طبيعية في جهاز المناعة تستخدم لتنظيم استجابته وضع الهجمات المفرطة على أنسجة الجسم السليمة

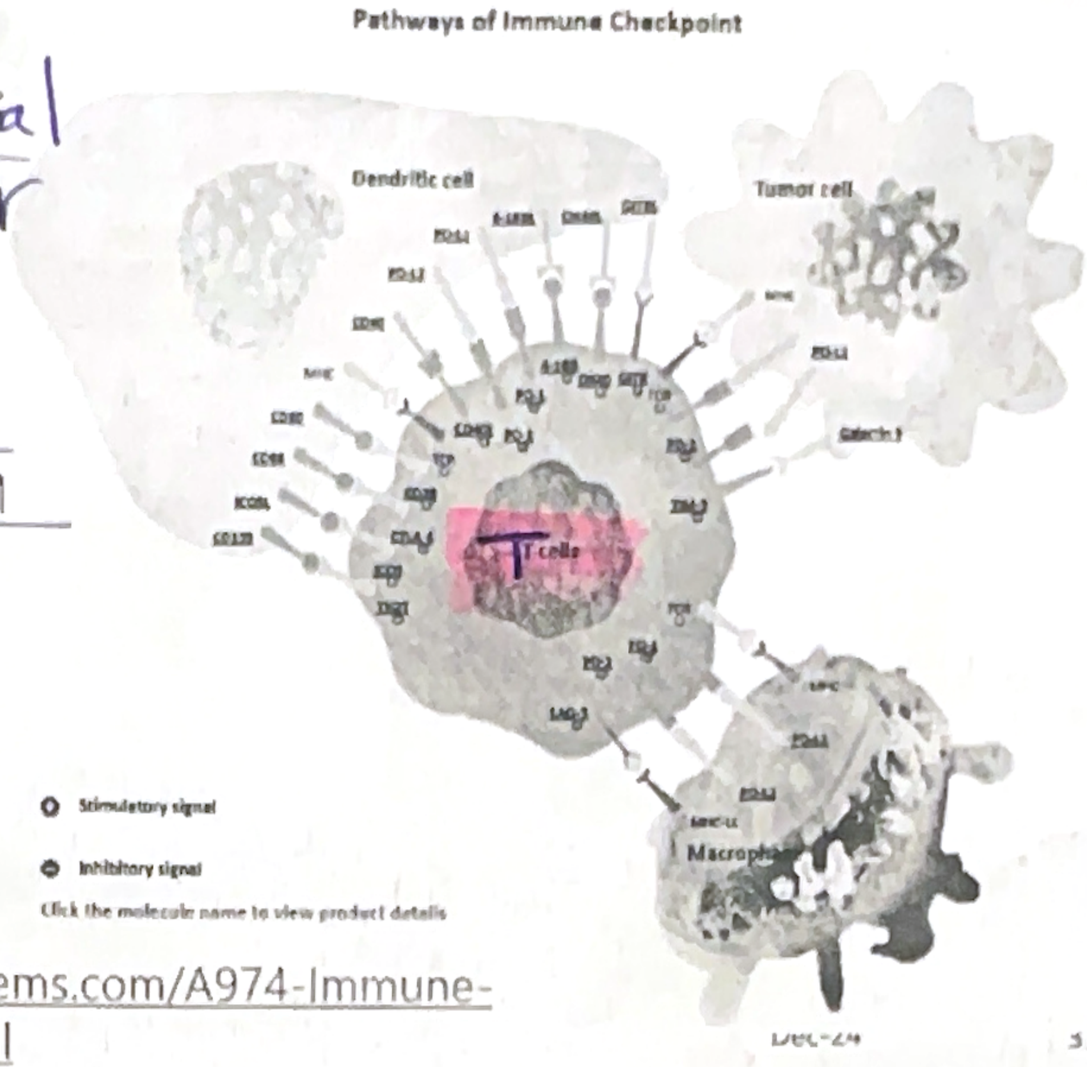
بعض الهدف منها أيضا تحقيق توازن بين القضاء على مسببات الأمراض والأورام مع الحفاظ على الأنسجة السليمة ،

فالأورام السرطانية تستغل هاي نقاط التفتيش (immune check point) للهروب من الجهاز المناعي ، لذلك تم تطوير أدوية تنشط هاي النقاط (immune check point inhibitors)

لاستعادة قدرة الجهاز المناعي على التعرف على الخلايا السرطانية ومهاجمتها)

IMMUNE CHECKPOINTS

- Immune checkpoints are a normal part of the immune system. Their role is to prevent an immune response from being so strong that it destroys healthy cells in th body.



https://www.acrobiosystems.com/A974-Immune-Checkpoint-Proteins.html

بتفرضيكم الصورة كيف الخلايا بتعرفو على بعض من الجسم، كيف T cells لا تقتل خلايا الجسم الثانية (يكون في بينهم نوع من التواصل من خلال immune check points)

APPLICATIONS OF MONOCLONAL ANTIBODIES

(قرأتها عرادة)

- **Autoimmune Diseases:**
  - Rheumatoid arthritis (adalimumab).
  - Psoriasis (secukinumab).
- **Infectious Diseases:**
  - Respiratory syncytial virus (palivizumab).
- **Diagnostics:** Imaging and detection (e.g., radiolabeled antibodies).

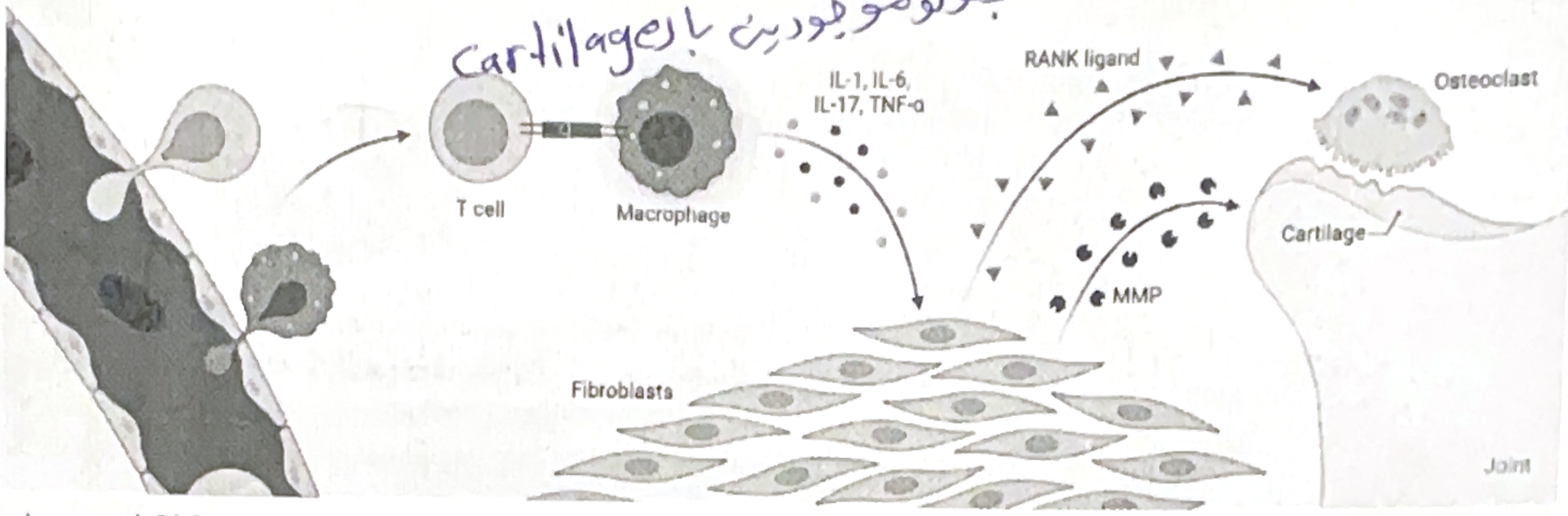
→ product approved by the F.DA to treatment of autoimmune disease like RA

Pathogenesis of Rheumatoid Arthritis

inflammatory factors بيصيرو active وبنتجو cytokines زي

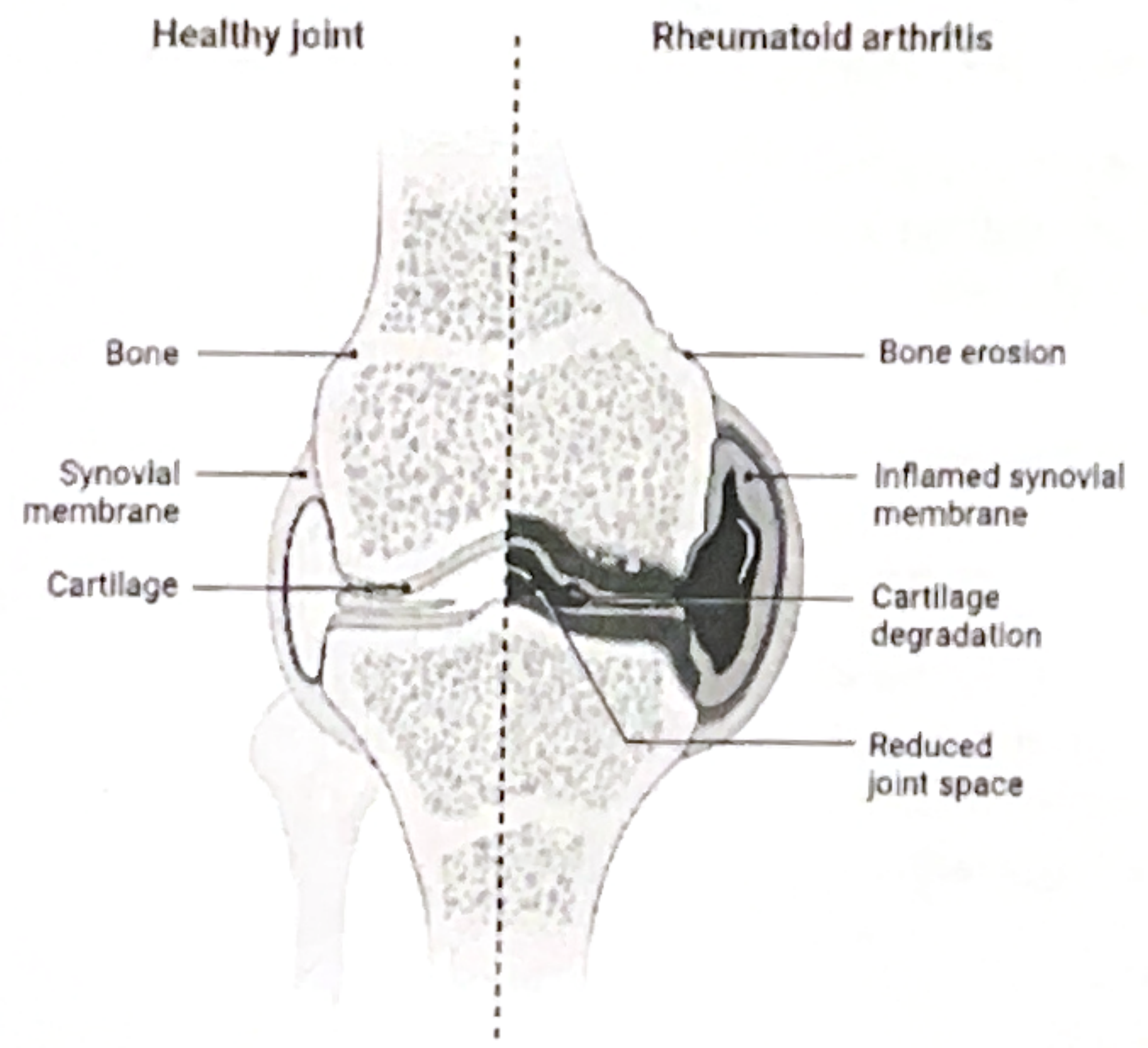
1. Unknown trigger promotes inflammation in synovial membrane, attracting leukocytes into the tissue
2. Autoreactive CD4 T cells activate macrophages, resulting in pro-inflammatory cytokine production
3. Cytokines induce MMP and RANK ligand production by fibroblasts
4. RANK ligand activates osteoclasts and MMP destroys tissue, resulting in joint destruction

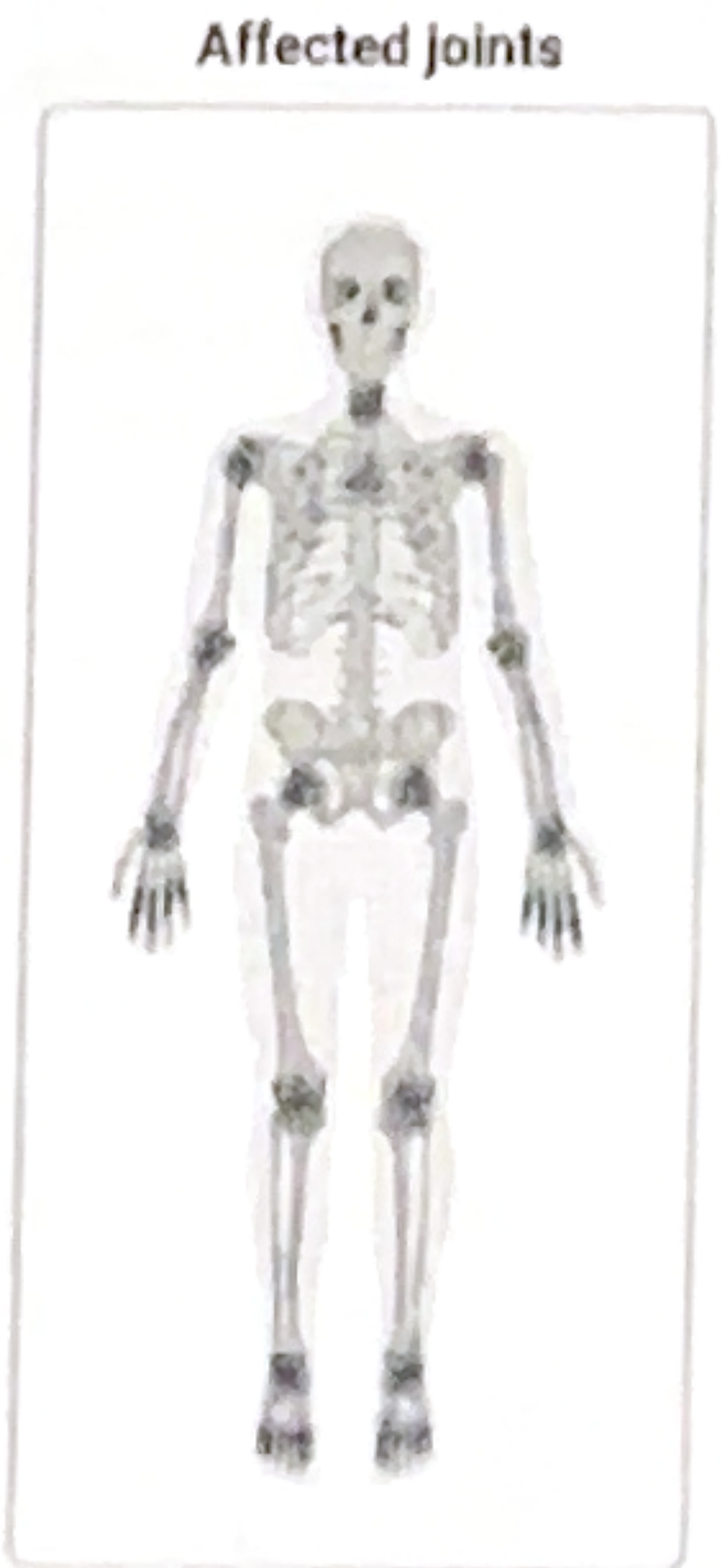
بيكونو موجودين بال cartilage



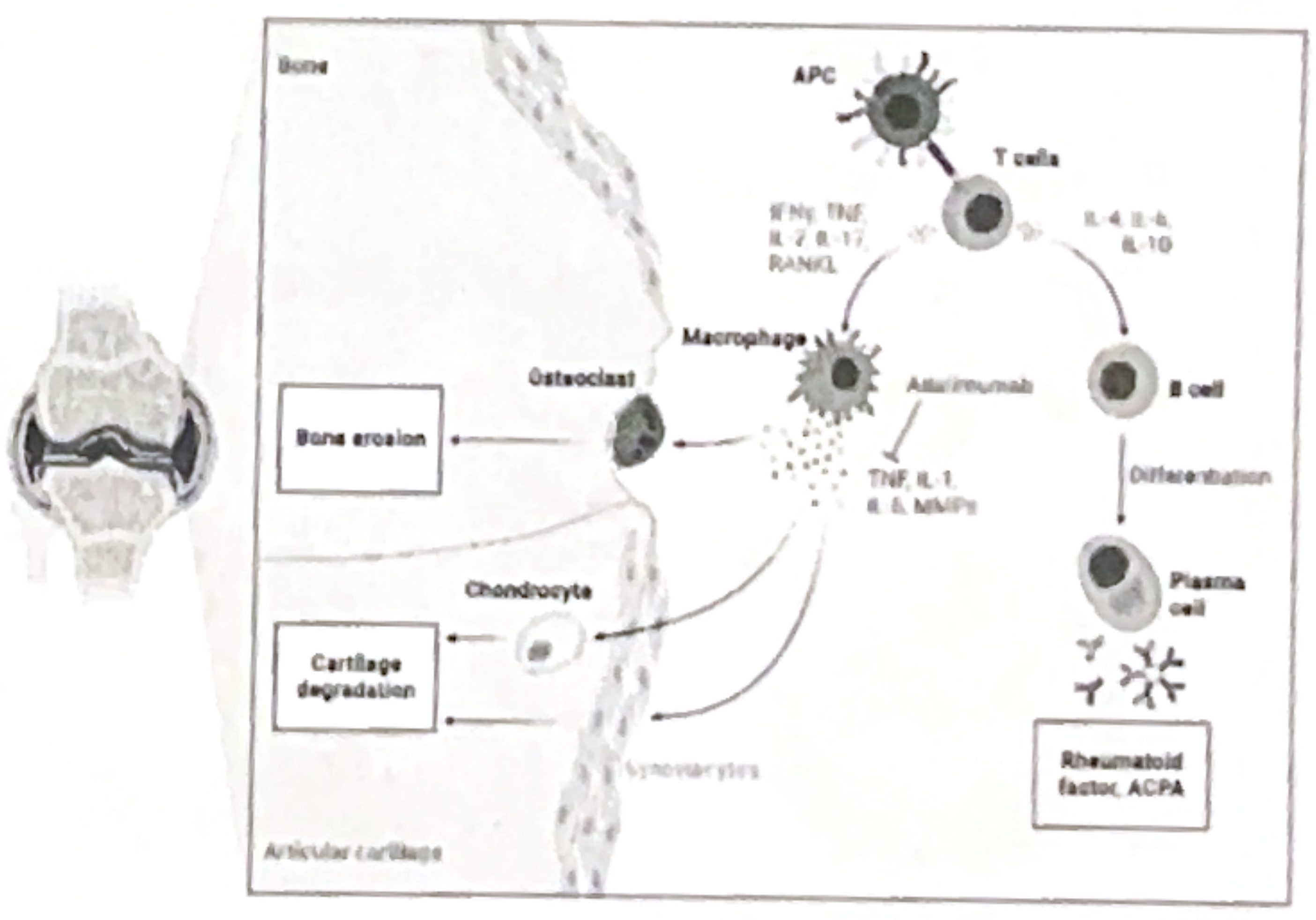
← عشان أمنع كل هاي process بعمل block لهدول الـ inflamatory factors (... TNF , IL-1 ، IL-6) ع من انهم يرتبطو في مكان ما عن fibroblast

adalimumab ← بعمل تثبيط لهاي العملية بالتالي بمنع osteoclast يهاجمو cartilage ويصيرلنا هاد التآكل لي بصير عند الناس يلي عندهم RA





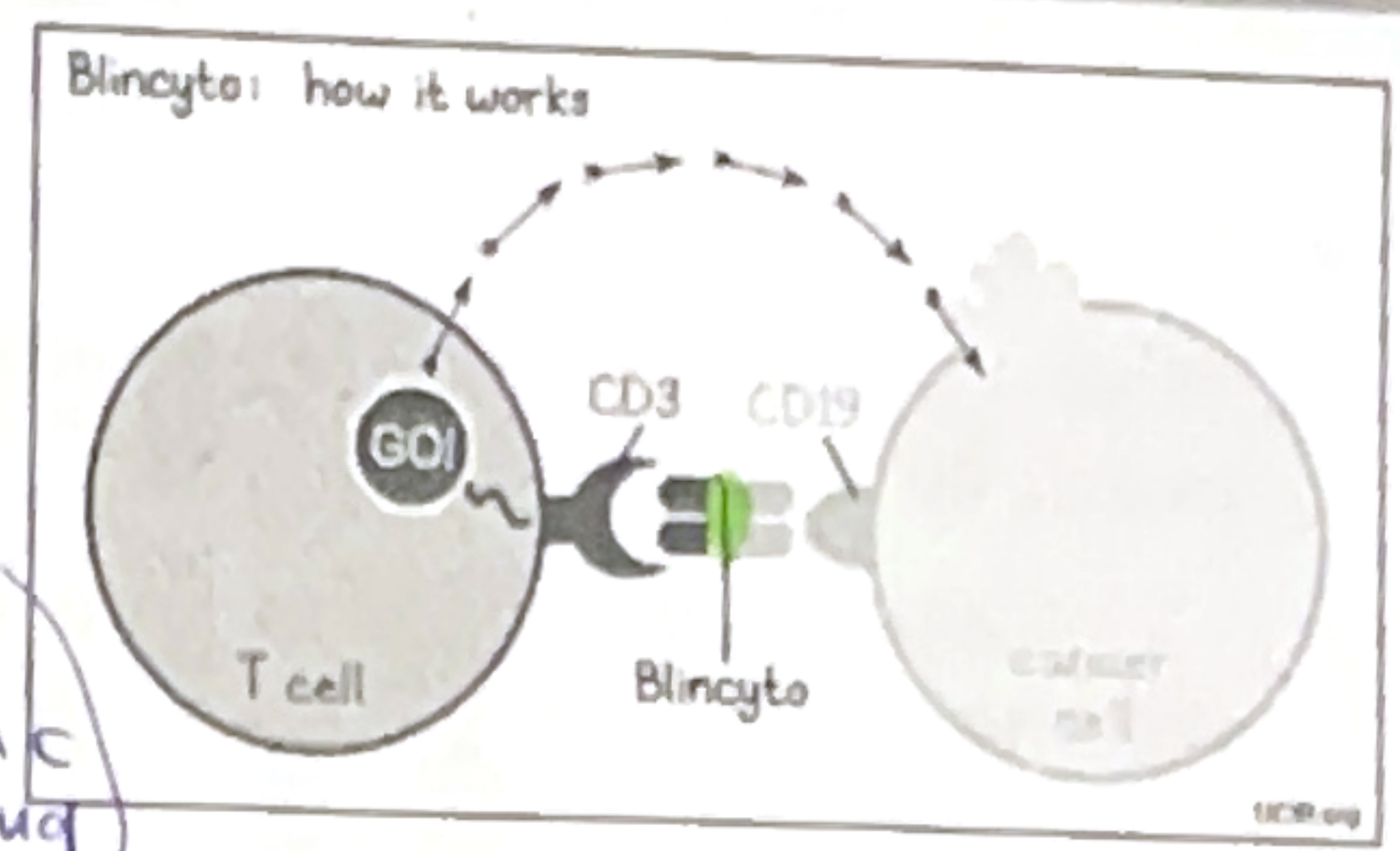
Site of Action of Humira (Adalimumab)

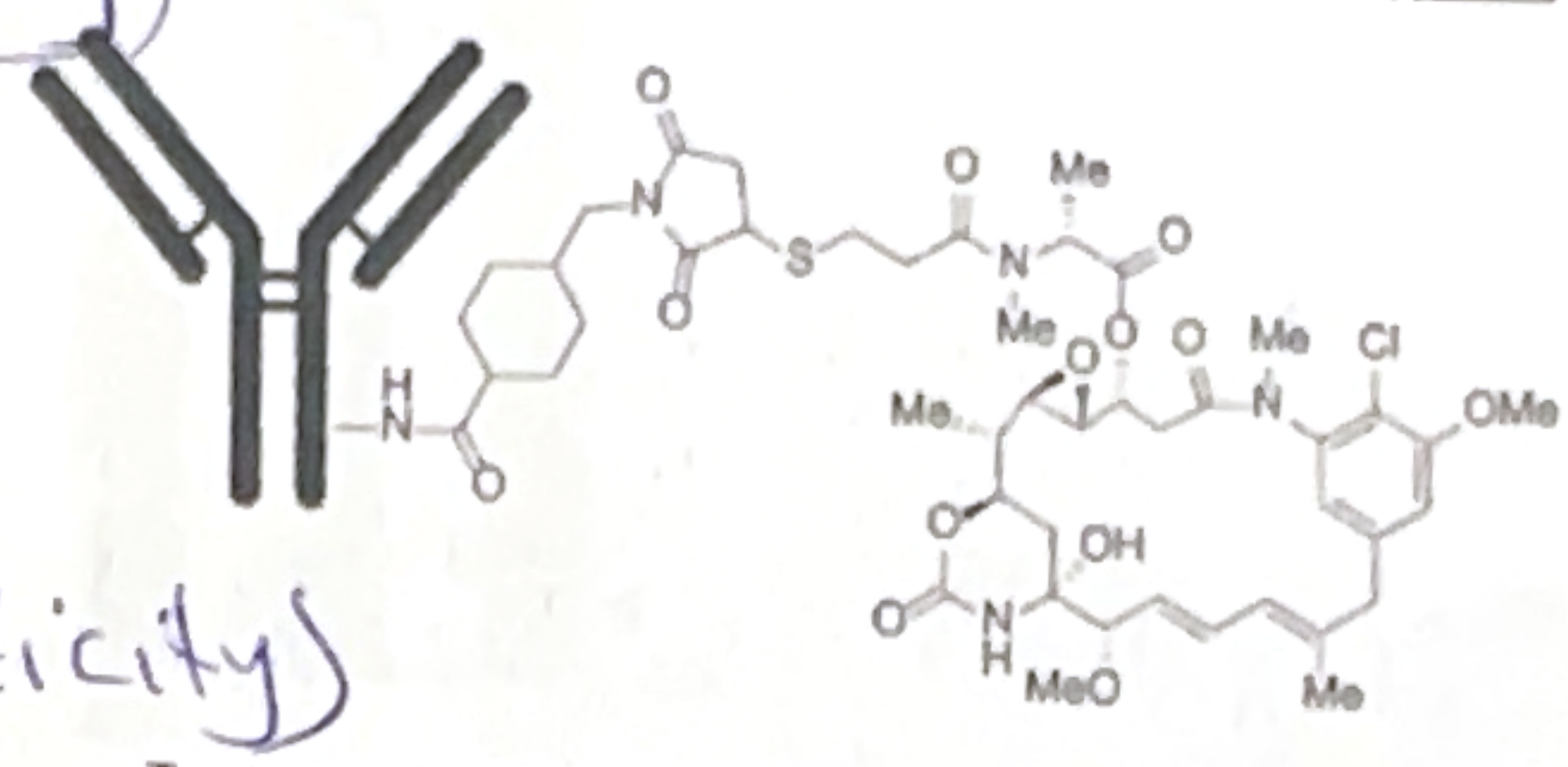


BISPECIFIC ANTIBODIES AND ADCS

- Bispecific Antibodies:
  - Dual specificity to two antigens (e.g., blinatumomab for acute lymphoblastic leukemia).
  - Mechanism: Bring immune cells closer to target cancer cells – cancer imunotherapy
- Antibody-Drug Conjugates (ADCs):
  - Combine cytotoxic drugs with antibodies (e.g., trastuzumab emtansine).
  - Targeted cancèr treatment, minimizing systemic toxicity.

(ربطوه بـ small molecule مثل: لتحسين cytotoxic effect)
↑ cytotoxic drug





Bispecific اسمه mono AB نوع من ال ←
(dual specificity) يعني بقدرو يرتبطو بنفس الوقت

ميكانيزم، يرتبطو هدول ال AB ال Cancer cell of interest
زي كأنهم بعملو tag لل cancer cell بتحكي للـ Tcell
تعالي أنا هون، فبتيجي T-cell تهاجم Cancer cell
وتقتلها (هاي AB تقتل الخلايا السرطانية بطريقة غير مباشرة، بتخلي الجسم هو لي يقتلها)

FEW COMMERCIALLY AVAILABLE mAbs APPROVED BY FDA

(ما شرحته)

Name	Trade name	Target	Use
Abciximab	ReoPro	CD41 (integrin alpha-IIb)	Platelet aggregation Inhibitor
Adalimubab	Humira	TNF-*alpha*	Rheumatoid arthritis, Crohn's Disease, Plaque psoriaris, psoriatic Arthritis
Alirocumab	Praluent	PCSK9	Hypercholesterolemia
Avilumab	Bavencio	PD-L1	Cancer
Benralizumab	Facenra	CD125	Asthma
Daclizumab	Zenapax	CD25	Organ transplant rejection
Daratumubab	Darzalex	CD-38	Multiple Myeloma

EVALUATION OF mAbs

(قراءة)

- Evaluation
- "Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products", WHO, 1st March, 2016
- • "Guideline on development, production, characterisation and specification for monoclonal antibodies and related products", EMA, 21st July, 2016

EVALUATION OF mAbs

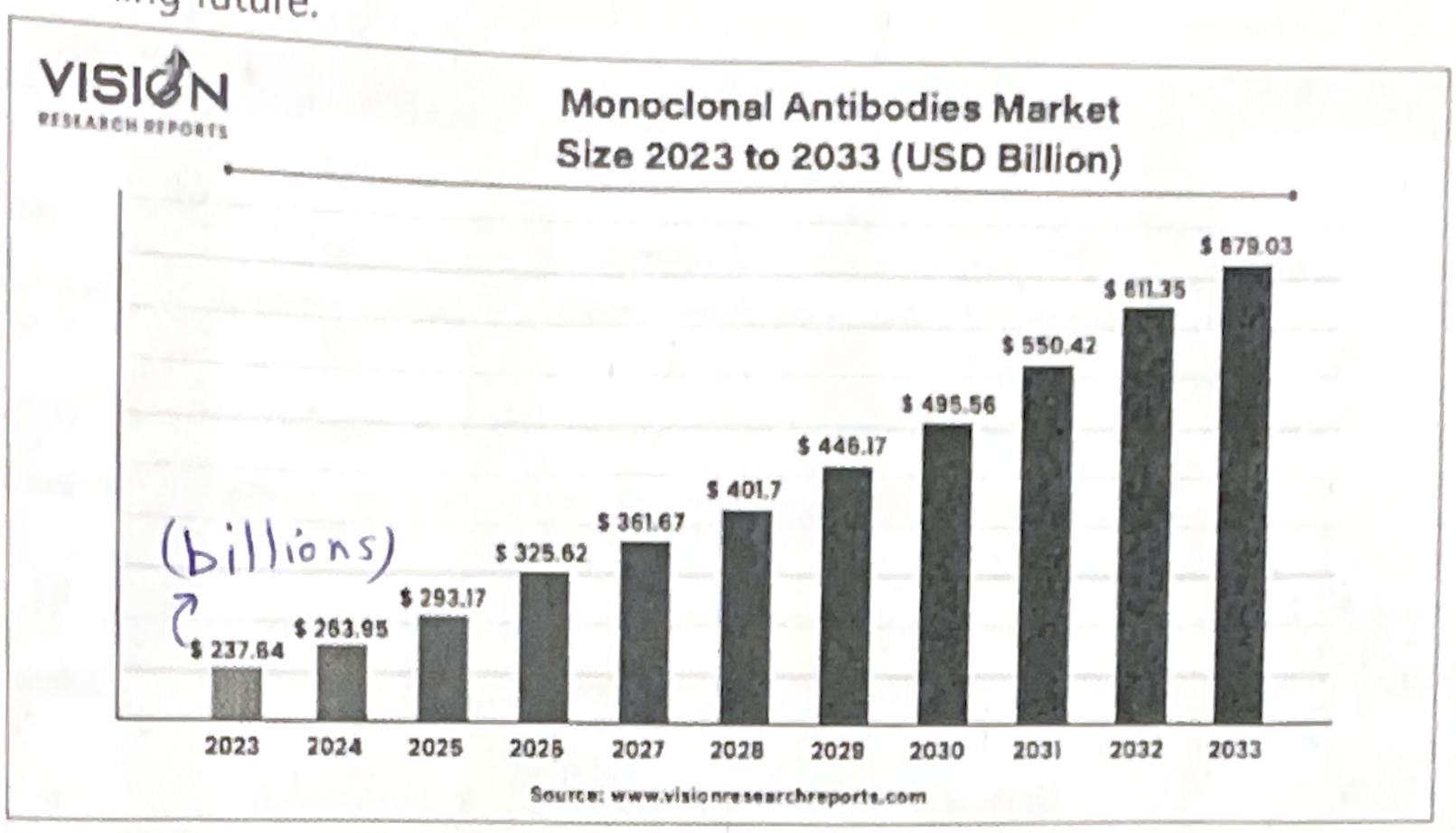
سو بنفحص بال evaluation

- **Characterisation of monoclonal antibodies**
  - Physicochemical characterisation
  - Immunological properties
  - Biological activity
  - Purity, impurity and contaminants
  - Quantity
- **Specifications**
  - Identity
  - Purity and impurities
  - Potency
  - Quantity
  - General tests → عب سئو

ال application، وعب نوع AB

GROWTH OF mAbs MARKET

- Vast growing number of approved product in the market will increase the incidence of monoclonal market in the coming future.

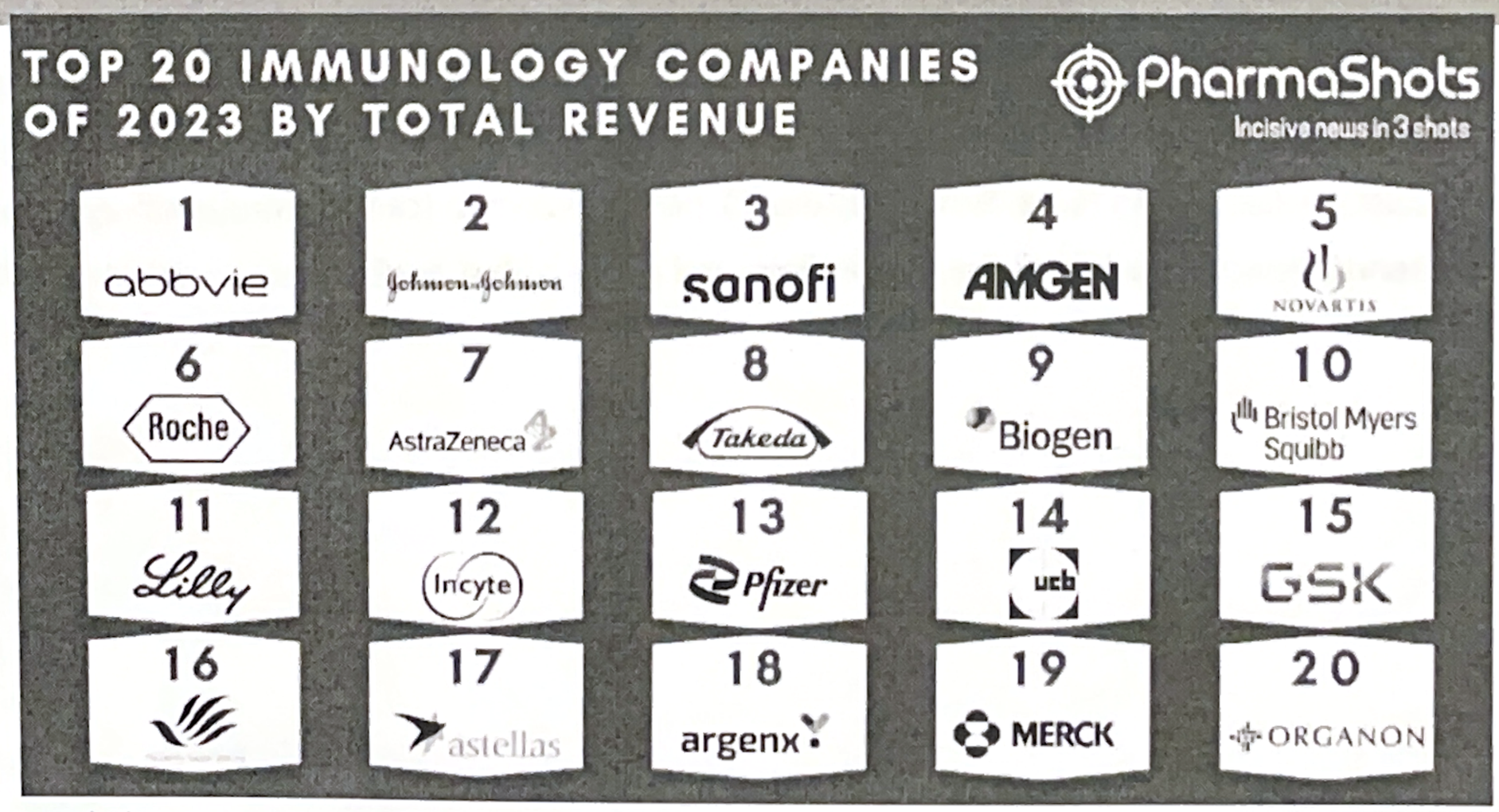


تصنيع هاي AB بتارع وتكبر بالسوق العالمي بشكل كبير، بتنصحكم تقرأوا عنه

MARKET TRENDS FOR PROTEIN DRUGS AND MAB

- **Global Growth:** Biologics dominate the pharmaceutical market, with sales exceeding $300 billion annually.
- **Top Products:** Adalimumab (autoimmune diseases), pembrolizumab (cancer immunotherapy).
- **Future Trends:** Personalized medicine, biosimilars, and gene-edited antibodies.

تحقق أرباح طائلة



BIOSIMILARS

- **Definition:** Biologic medical products highly similar to reference biologics.
- **Importance:**
  - Cost-effective alternatives.
  - Increase access to life-saving therapies.
- **E.g.** Trastuzumab-dkst, infliximab-dyyb.
- **Regulatory Pathways:**
  - Require robust analytical, preclinical, and clinical data.

Feature	Trastuzumab	Trastuzumab-dkst
Type	Original biologic drug	Biosimilar of trastuzumab
Brand Name	Herceptin ®	Ogivri ®
Developer	Genentech/Roche	Mylan & Biocon
Approval Basis	Full clinical trials to demonstrate safety and efficacy	Demonstrated high similarity to trastuzumab with no clinically meaningful differences
Cost	Higher (innovator drug)	Lower (biosimilar option)
Manufacturing	Proprietary process with original cell lines and detailed process optimization	Independent reverse engineering to match structure, function, and glycosylation patterns
Production Method	Produced using genetically engineered mammalian cells (CHO cells)	Similar mammalian cell expression systems (typically CHO cells), but independently developed processes
Structure and Function	Identical HER2 receptor targeting	Highly similar targeting and mechanisms
Indications	HER2-positive breast and gastric cancers	Same as trastuzumab
Market Availability	Longer market history	Approved as a cost-effective alternative
Regulatory Pathway	Biologics license application (BLA)	Biosimilar approval pathway

originator – generic نفس فكرة original biologic – bio similars

بصنعوهم كتير بشبهو ال original لكن بروحو الشركات بتجاه تصنع biosimilars ؟ لانهم ارخص لانهم ببساطة ما بحتاجو يرجعو يعملو كل research, approval, Validations ... بتكون كتير مختصرة مقارنة بالشغل يلي اشتغلوه الناس لل original

INNOVATIONS IN DELIVERY SYSTEMS

ما شرحتها (راح نوخدهم لقدام soon)

- **Nanoparticles:** Protect proteins from enzymatic degradation and enable targeted delivery.
- **Microneedles:** Painless delivery system for vaccines and biologics.
- **Liposomes:** Encapsulation enhances bioavailability and stability.
- **PEGylation:** Increases half-life and reduces immunogenicity.

وكمان لما تنتهي ال License ال product original ويسمحو للباقي الشركات انه يصنعوه كمان بصير في تنافس كبير بينهم فالسعر للمنتج بقل

ETHICAL CONSIDERATIONS

biotechnological كل ما يخص application

- **Accessibility:** High costs limit availability in low-income regions.
- **Equity:**
  - Balancing innovation with affordability.
  - Ensuring global distribution of biologics.
- **Sustainability:** Environmental impact of large-scale biologics manufacturing.

أغلى أدوية + large scale بتقدر أصنعه بـ

للبيئة

+

revenue أعلى

REGULATORY AND QUALITY ASSURANCE

قراءة

- **Regulatory Oversight:**
  - FDA and EMA guidelines for biologics.
- **Quality Control:** Stability, potency, and absence of contaminants.
- **Risk Management:** Identifying and mitigating immunogenicity risks.

FUTURE DIRECTIONS IN PROTEIN THERAPEUTICS

مراجعة

- **Advances in Engineering:** Next-gen mAbs (bispecific, trispecific antibodies).
- **Personalized Medicine:** Tailored therapies based on patient genetics.
- **AI Integration:** Enhancing biologics discovery and optimizing manufacturing.
- **Sustainability:** Innovations to reduce environmental footprint in production.