Gene therapy

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Gene therapy (المعالجة الجينية)

- **Gene therapy** is the use of **nucleic acids** as therapeutic medicinal compounds for a diseases which have **limited** or no **therapeutic options** (للأمراض محدودة أو معدومة الخيارات العلاجية).
- **The gene therapy uses different strategies:**

o Gene therapy could moderate **the abnormal gene expression** (تعديل التعبير الجيني) .

o Gene medicines can also be engineered **to reconstitute a diseased organ** (اعادة ترميم العضو المريض) .

o **Regeneration** (اعادة توليد) of specific tissues through expression of embryonic genes to induce cell growth and development.

o using natural or genetically corrected **stem cells** (خلايا جذعية) to produce healthy tissues

Gene therapy and their application (تطبيقات المعالجة الجينية)

- In **adenosine deaminase** (ADA) deficiency (عوز أنزيم), by using gene therapy involved the use of peripheral blood lymphocytes (خلايا لمفاوية من الدم المحيطي)treated with a **retrovirus expressing ADA (يعبر عم الأنزيم)** in ADA-deficient patients (Anonymous, 1990).

Disease	Gene therapy clinical trials	
	Number	Percentage
Cancer	842	67.0
Vascular diseases	113	9.0
Monogenetic diseases	104	8.6
Infectious disease	81	6.4
Gene marking	50	4.2
Healthy volunteers	21	1.7
Other diseases[a]	47	3.7

[a] Grouped in this category are treatments for: inflammatory bowel disease, rheumatoid arthritis, chronic renal disease, carpal tunnel syndrome, Alzheimer's disease, diabetic neuropathy, Parkinson's disease, erectile dysfunction, retinitis pigmentosa and glaucoma.
Source: From Anonymous, 2006 and Edelstein, 2004.

EX VIVO versus IN VIVO gene therapy

- In the disease treatment using **gene therapy** there are many aspects have to be considered (هناك العديد من المفاهيم يجب اخذها بعين الإعتبار) :

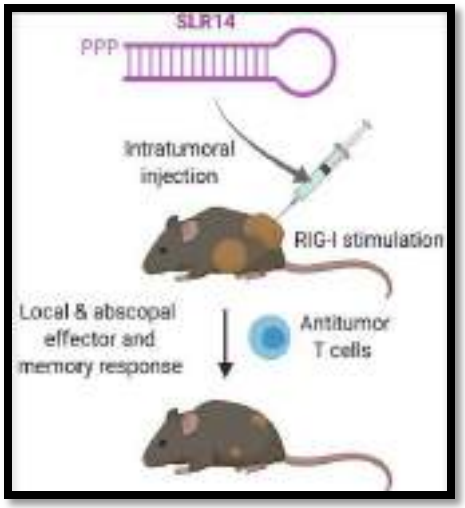
  - The **gene** necessary for treatment has to be **identified and cloned (تحديد و تنسيل)** .

  - The **disease** and the **gene product** have to be well understood (دراسة المرض و المنتج الجيني بشكل جيد) in order to insure that the **therapeutic components** delivered to the **appropriate cellular compartment** responsible for its processing and subsequent biological activity (الفعالية البيولوجية الناتجة عن المعالجة) .

  - Developing **immune response** to the **gene transfer** system (الإستجابة أو رد الفعل المناعي لنظام النقل الجيني) could be a problem in this area of medicine

Several stratigies can be used for gene transfer (يمكن استخدام العديد من الإستراتيجيات لنقل الجين)

- **Direct injection (الحقن المباشر)** of **vector/ DNA** complexes into the **bloodstream** is often characterized by **low levels of gene expression** Broad distribution of the vector could be correlated with **side effects**.
- **Intratumoral (حقن داخل الورم)** , **intraperitoneal (داخل الصفاق أو الغشاء البيريتواني), subcutaneous(تحت الجلد)**, and **intramuscular** injection.



EX vivo gene transfer :

- involves **isolation** and **culture** of cellular targets (عزل و زرع الخلايا الهدف).
- Gene transfer is achieved by direct application of the vector (virus, plasmid) for efficient gene expression.
- only **healthy cells** expressing the therapeutic **gene collected** and given to the patients (يتم فقط نقل الخلايا المعبرة عن البروتين العلاجي).
- In the **Ex- vivo gene transfer** is safer because the host immune response to the vector or the **toxic effects** associated with the transfection reagents **are eliminated**.

Disease target

- There are currently **1.260** active gene therapy **clinical trials** world wide Approximately **67%** of these trials are **for cancer.**

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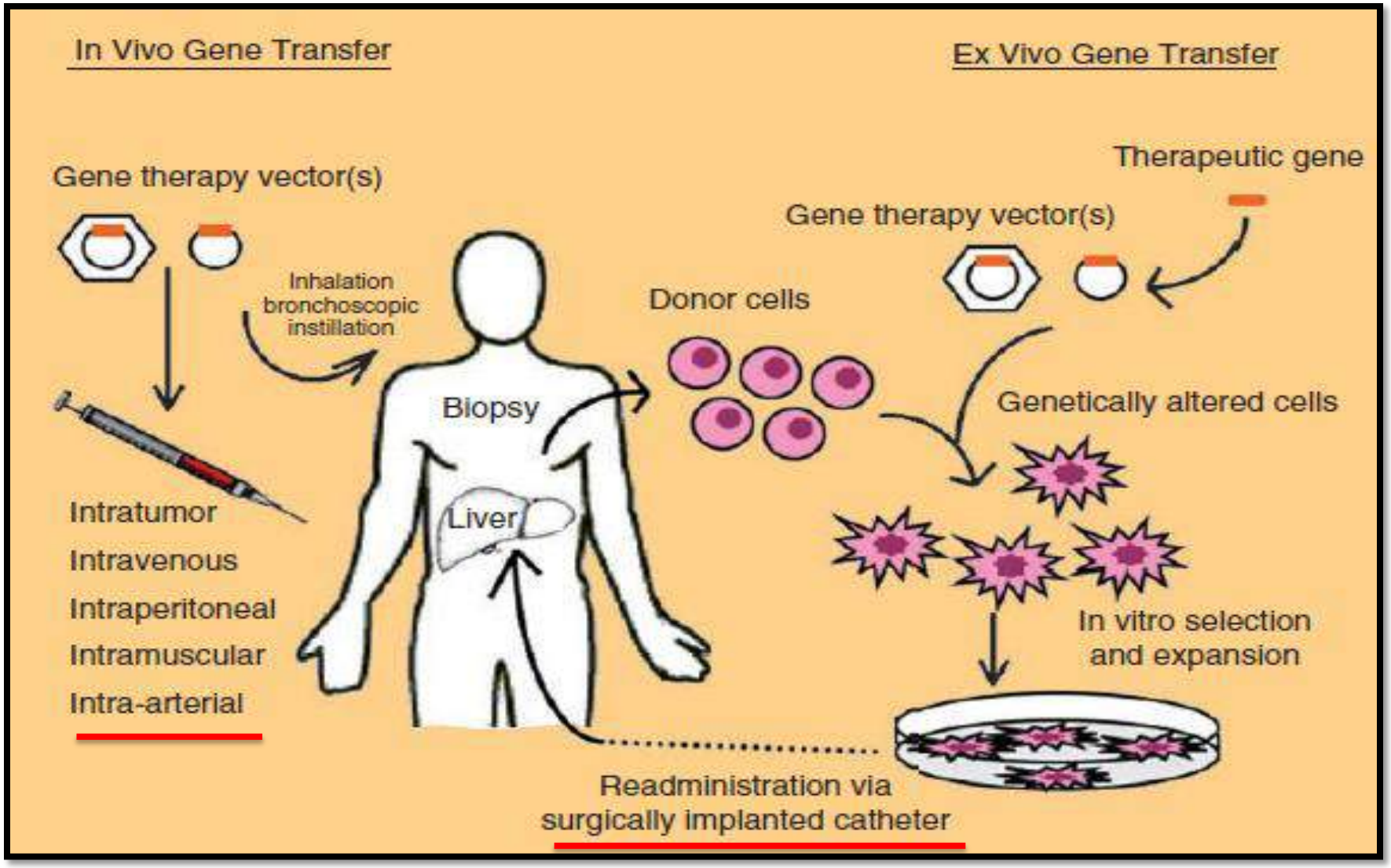
GENDICINE:
- p53 gene + adenovirus
- gene therapy for cancer
- medical breakthrough

The first gene therapy product is approved. On October 16, **2003, China's SFDA** approved an adenovirus-based product, **Gendicine,** for treatment **of head and neck cancer.** The product was commercially available in January 2004 through the company **SiBiono GeneTech**

In/Ex vivo gene transfer



Gene therpy for cancer

- The aim of applying the gene therapy is to **destroy tumor (تحطيم الورم)**cells and **preserve** the **normal tissue.**

- **Many strategies** could be considered in cancer gene therapy:

- **correction of genetic mutations (تصحيح الطفرات الجينية المرافقة للنمط الورمي)**contributing to the malignant phenotype.
- **stimulation** of a **T-cell-mediated immune** response against the tumor (immunotherapy).
- use of **oncolytic viruses (فيروسات حالة للورم)** that replicate in and destroy **tumor cells** (**virotherapy**) (معالجة فيروسية)
- use of **enzyme pro-drug systems (انظمة الأنزيم طليعة الدواء)** that **destroy tumor cells** by **converting** a non-toxic medicinal compound to **cytotoxic metabolites (مستقلبات سامة).**

Gene therpy for cancer

Correction of genetic mutations (تصحيح الطفرات الجينية):

- Approximately **12% of cancer gene** therapy clinical trials involve over-expression of **tumor suppressor genes (الجينات المثبطة للورم)** such **as p53,** MDA-7 and ARF.
- First approved **gene therapy recombinant adenovirus** expressing **this transgene, Gencidine**, by China's State Food and Drug Administration (SFDA) making it the first gene therapy product available for worldwide clinical use.

Immuno-therapy:

- Stimulating the **anti tumor immune** response (الإستجابة المضادة للورم) could be achieved by Expression of **pro-inflammatory cytokines** (interleukin (IL)-2, and IL-12).

Gene therpy for cancer

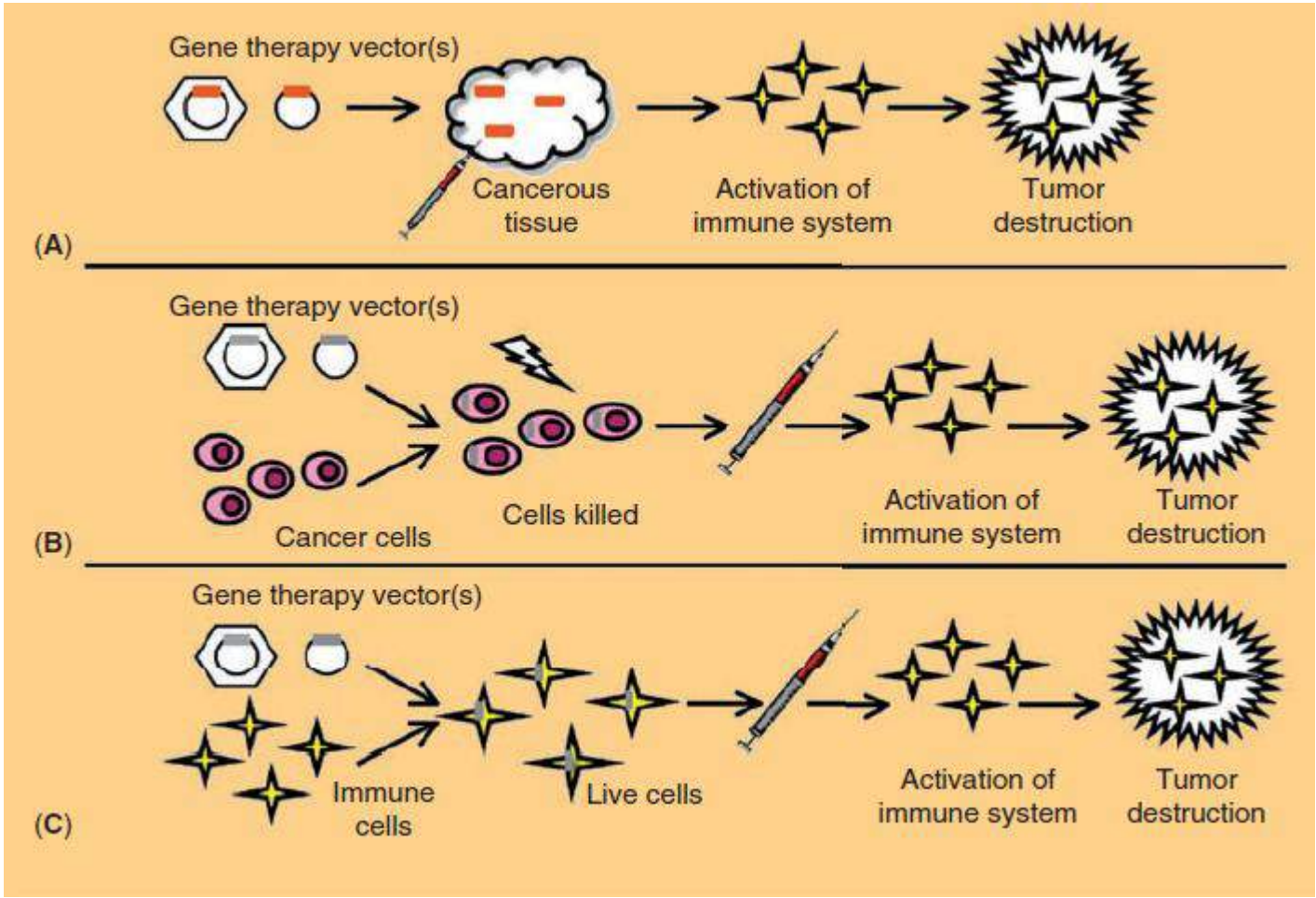
- **Efficient removal of malignant tissue by the immune system can be achieved by:**

o **Direct injection (حقن مباشر)** of a vector expressing immunostimulatory molecules or tumor-specific antigens (مستضدات نوعية للورم) in a **tumor.** As the **transgene product is released**, macrophages, dendritic cells, natural killer cells and T-cells are activated and migrate to the tumor where they **destroy cells expressing the transgene**.

o **Cells isolated from** the patient or cancerous cell lines are treated with the vector **in culture, killed by irradiation** and given back to the patient. **Epitopes** (حواتم)on the cells prompt the immune system to attack and **remove malignant cells تحفز) الجهاز المناعي لتهاجم و تنزع الورم)**

o **T-cells or bone marrow** cells from the patient are **cultured** with a **vector** and/or **tumor antigens**. **Live cells** that attack and remove malignant cells are **given back to the patient.**

Gene based immuotherpay for cancer

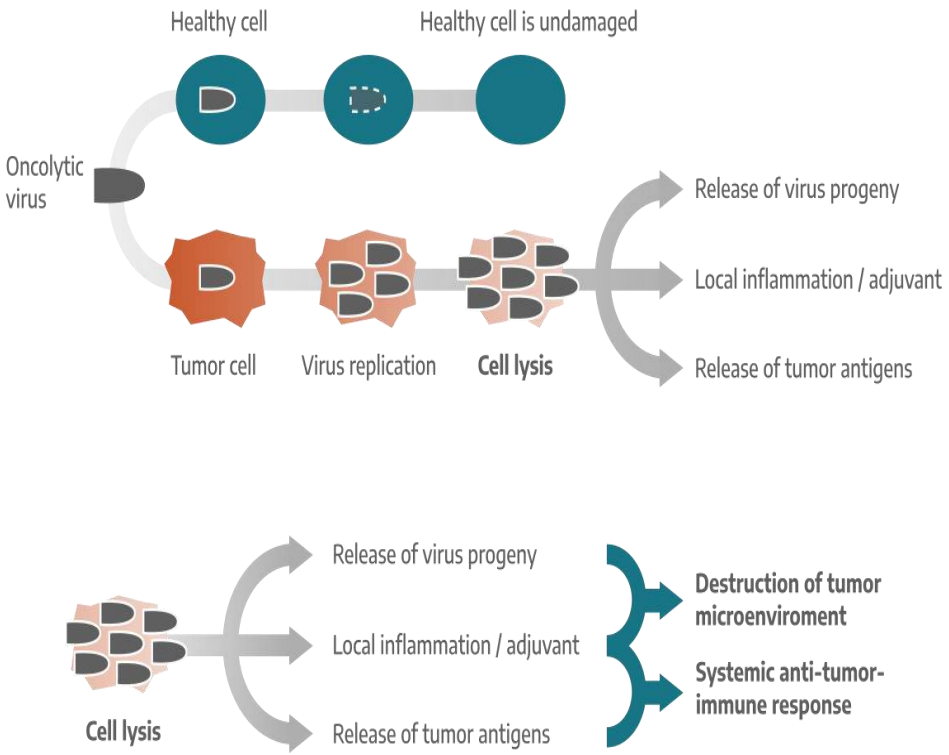


Oncolytic viruses (الفيروسات الحالة للورم) (virotherapy)

- **This viruses** induce tumor cell death through **replication**, **expression of cytotoxic proteins** and cell **lysis** in malignant cells while remaining unhurt in the rest of the body.

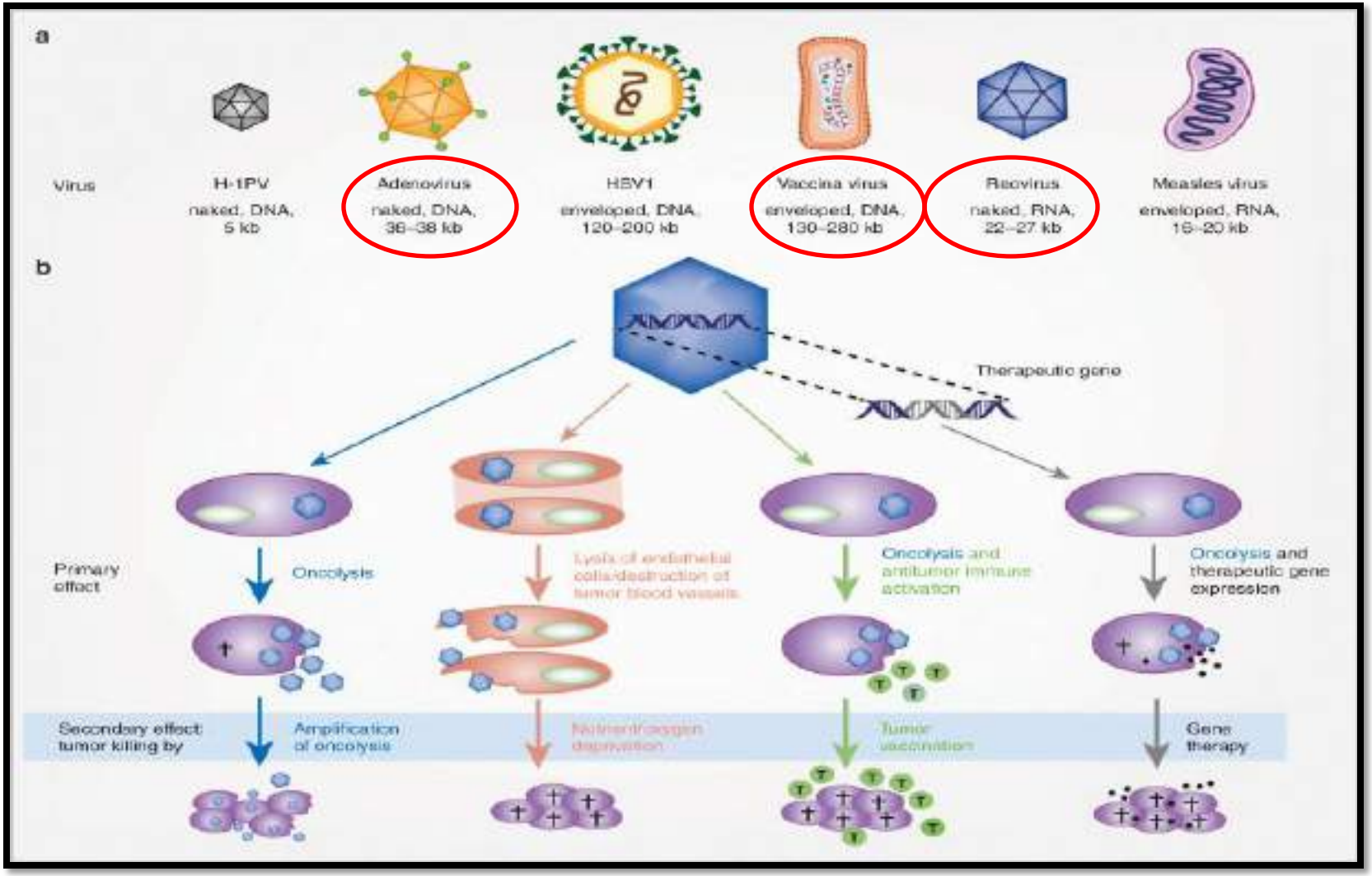
- **Vaccinia**, **herpes simplex type I (HSV),** r**eovirus** and **adenovirus,** often selected because naturally target cancer and have easily manipulated genome و (بشكل طبيعي تتوجه للخلية الورمية تمللك جين سهل المعاملة).

- **Disadvantag** that the many people have **naturally antibody** that clear the virus before replication and obtaining the effect.



Gene cancer therapy can improve the current treatment but more than one therapeutics necessary to achieve success.

Oncolytic viruses



Tumor snesitization (زيادة حساسية الورم)

- In this approach **genes are inserted** into the cancer cells to make them **more sensitive** to the **conventional chemotherapy**, radio therapy or other treatments.

- **Transgene expression of the p53** sensitize the tumor cells to the therapeutic effects.

- Also to overcome **MDR multi drug resistance (المقاومة المتعددة للأدوية الأورام)**in cancer . MDR represents **P-glycoprotein**, drug efflux transporter of cancer cell membrane.

- **siRNA** or vector **mediated MDR1 gene** silencimg were widely reported to be successful to reduce **chemoresistance of certain types of cancer**

Gene directed enzyme prodrug therpay (GDEPT)

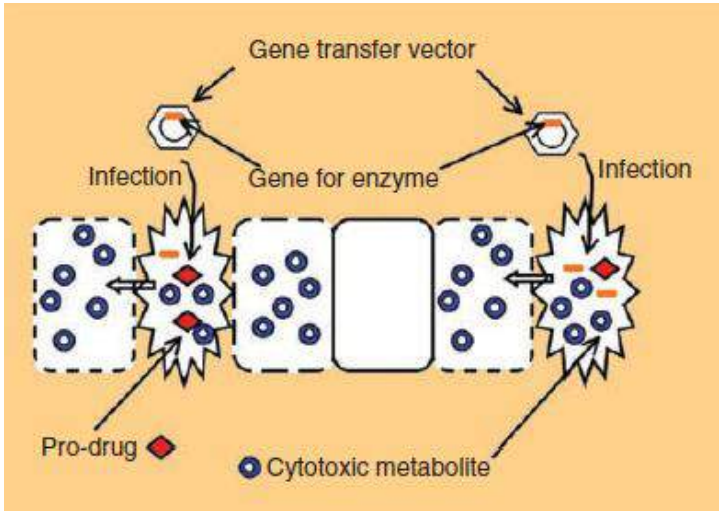
- One of the primary goals of cancer therapy (من الأهداف الأساسية لعلاج الأورام)is to deliver **highly potent**, **cytotoxic** compounds to **tumors and mestastases** and **limiting the exposure of normal** tissue to these agents**.**

- Gene encoding a **compound-specific enzyme** is delivered directly to tumor cells.

- The **corresponding prodrug** is given and is only converted to a **cytotoxic agent** by the **recombinant enzyme in the tumor.**

Gene directed enzyme prodrug therpay(GDEPT)

- Some **GDEPT** strategies (البعض من استراتيجيات هذه الطريقة)rely on a "bystander effect," where cytotoxic agents produced by transfected cells spread to **surrounding cells** for arrest and regression of tumor growth.

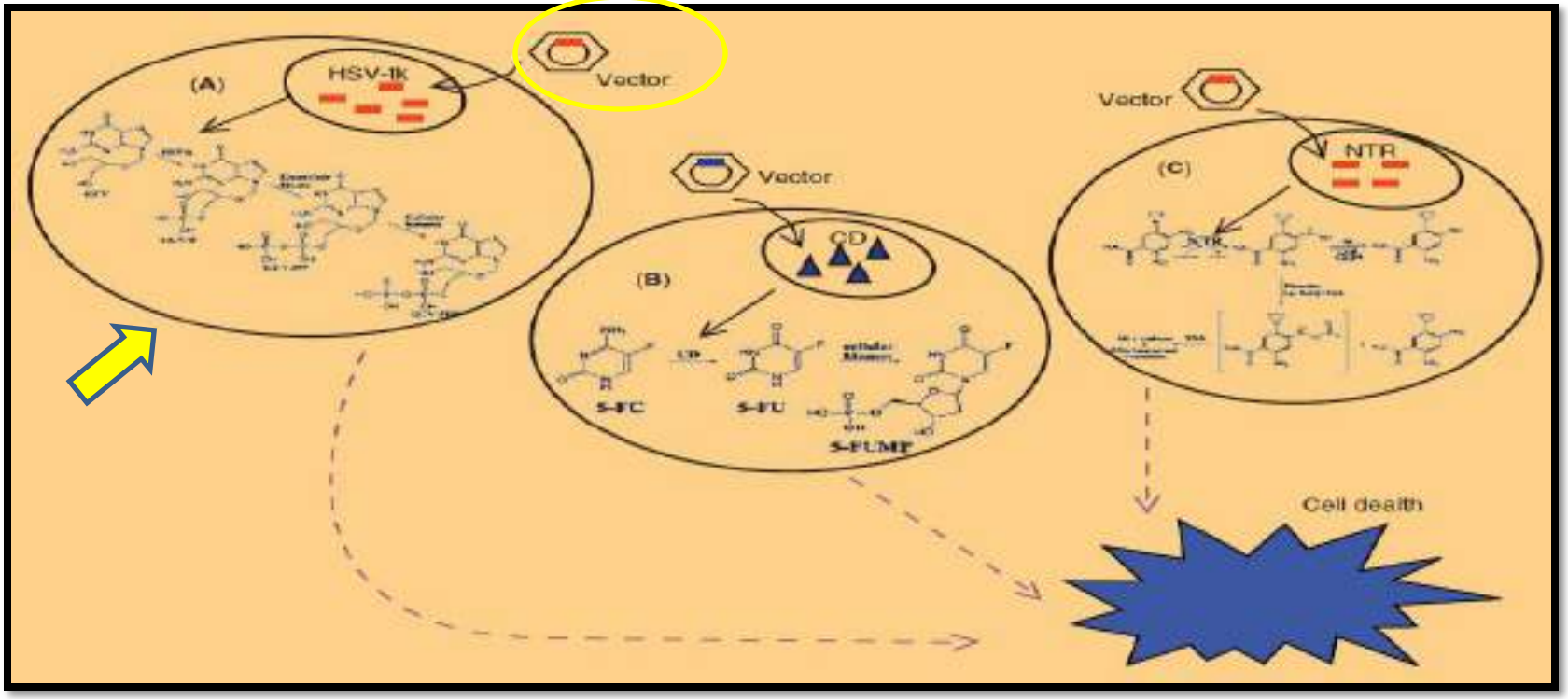


- A standard example of the **GDEPT** is Over-expression of the **herpes simplex virus thymidine kinase (HSV-tk)** gene with **gancyclovir**.

- This system is **selective** because **gancyclovir**, a poor substrate for human **monophosphatase kinase**, is rapidly converted to the **triphosphate form** after **phosphorylation** in a cell expressing **HSV-tk.**

Gene directed enzyme prodrug therpay(GDEPT)

- The **triphosphate** competes (ينافس) **deoxyguanosine triphosphate** during **DNA elongation (عملية تطاول سلسة الدنا)** and, once incorporated in a strand, **blocks DNA polymerase** and induces single strand **breaks (كسور في الدنا).**
- **HSV-tk/ gancyclovir** they only **GDEPT** reached **Phase III** clinical trials.



Vascular disease gene therpay (المعالجة الجينية للأمراض الوعائية)

- **Over-expression** (فرط التعبير)of genes involved in **vasodilation** (الجينات المسؤولة عن التوسع الوعائي)such as **endothelial nitric oxide synthase (eNOS)** have reduced blood pressure in animal models of **hypertension** (ارتفاع الضغط).

- This method have a long term control and overcome the **patient non compliance** (عدم مطاوعة المريض).

- **Over-expression** of genes that can **reduce cholesterol** (الجينات التي تستطيع خفض الكوليستيرول)such as apoproteins ApoA-1 and ApoE and the low density lipoprotein **(LDL)** and very low density lipoprotein **(VLDL) receptors** have been used for the treatment of inherited disorders of **lipid metabolism** (استخدمت لعلاج الإضطرابات الوراثية للإستقلاب الشحوم).

Vectors for gene transfer (حوامل لنقل الجين)

- Gene therapy can **be classified** to **non viral** and **viral gene** therapy
  (تقسم المعالجة الجينية الى معالجة جينية فيروسية و غير فيروسية) .

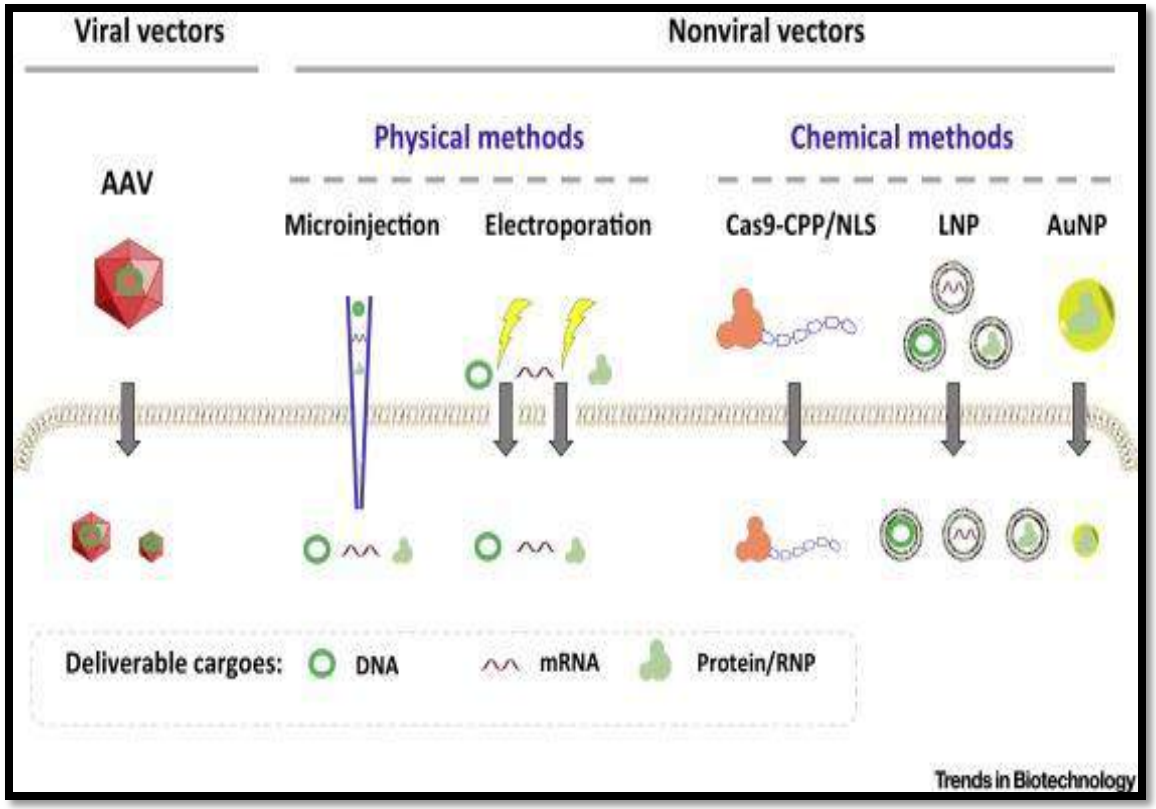
- Both of which rely on the successful construction of **gene expression plasmid (تعتمد على تصنيح ناجح لبلاسميد تعبير جيني) .**

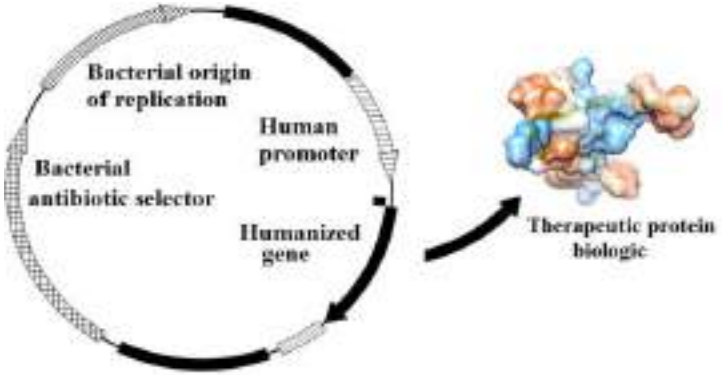
- The **plasmid** is a circular double strand DNA molecule (دنا حلقي مضاعف الطاق) which contain complementary **DNA (cDNA)** sequence coding for therapeutic gene .

Vectors for gene transfer

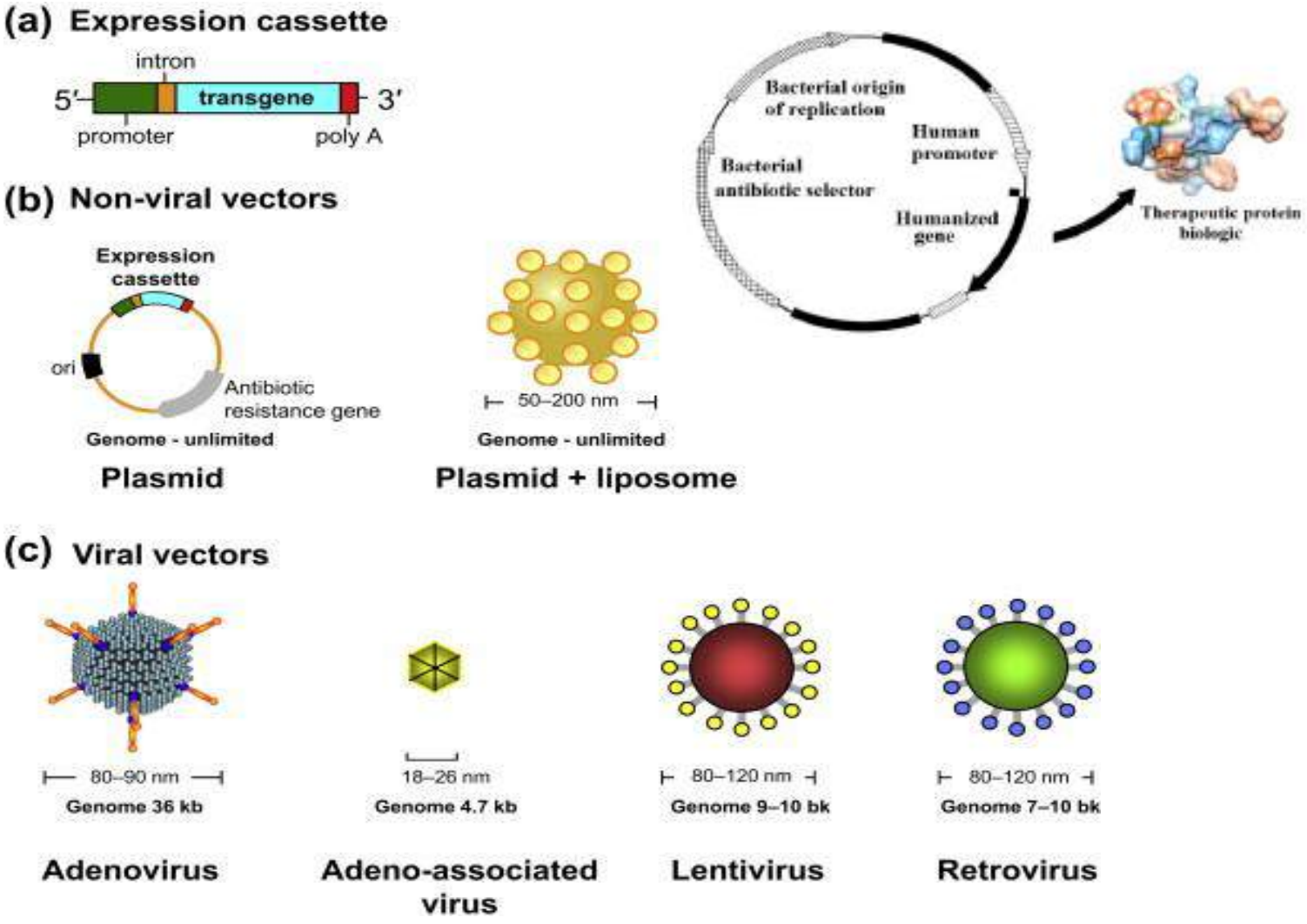
- Several **other genetic elements** (**يتضمن عناصر جينية اخرى**)including **bacterial elements** (**عناصر جرثومية**), **transcription regulatory** element (عنصر منظم للإنتساخ) **(TRE),** multiple cloning sites **(MSC),** untranslated region **(UTR),** introns, polyadenylation **(polyA),** sequences and **fusion tags** (**واصمات**)**.**

- Then many methods are used to validate the construct. Like s**equencing, PCR, immunoblott**.





Vectors for gene transfer



Production of gene transfer vector (انتاج حامل لنقل الجين العلاجى)

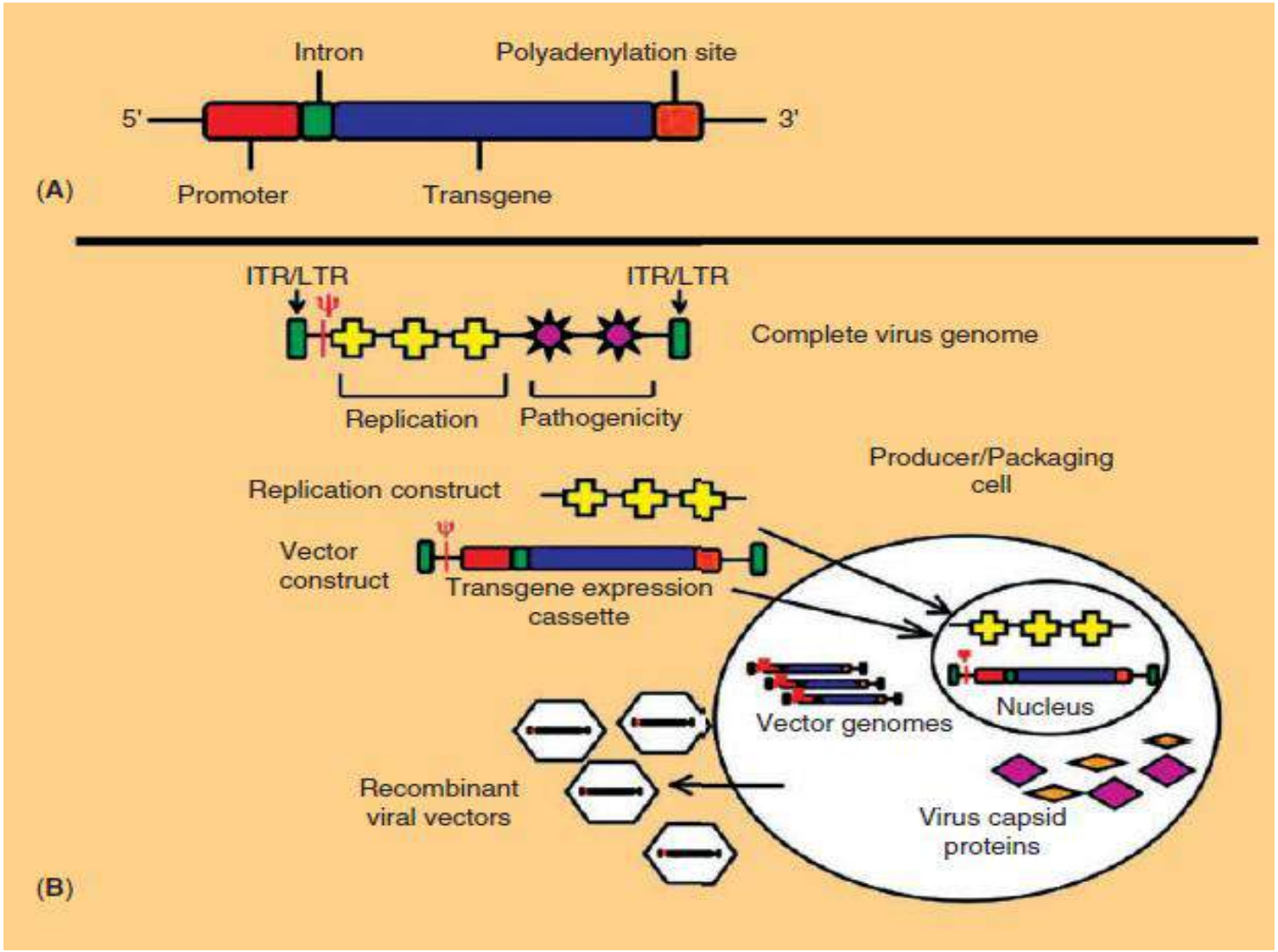
- (A) **Transgene expression cassette (تحضير كاسيت التعبير الجيني للجين العلاجي)**_. The therapeutic transgene cassette is bounded by a promoter at the 50 end and a polyadenylation site at the 30 end. This can be **cloned in a plasmid** and used directly for **gene transfer or it can be cloned** in a plasmid **containing viral elements** to produce **a recombinant virus** with the help of a **producer/packaging cell line**.

- (B) **Producer/packaging cell line**. (تحضير الخط الخلوي المنتج –المغلف) A packaging cell line is created by stably transfecting cells with a **plasmid containing genes needed for virus replication**. The **vector construct** often contains only **the packaging signal** (y) and the **transgene cassette flanked by the viral ITR/LTR sequences**.

- **Genes** responsible for **fulminant virus infection** are **removed** from the vector construct. The **vector construct** is introduced to the cell by **transfection**.

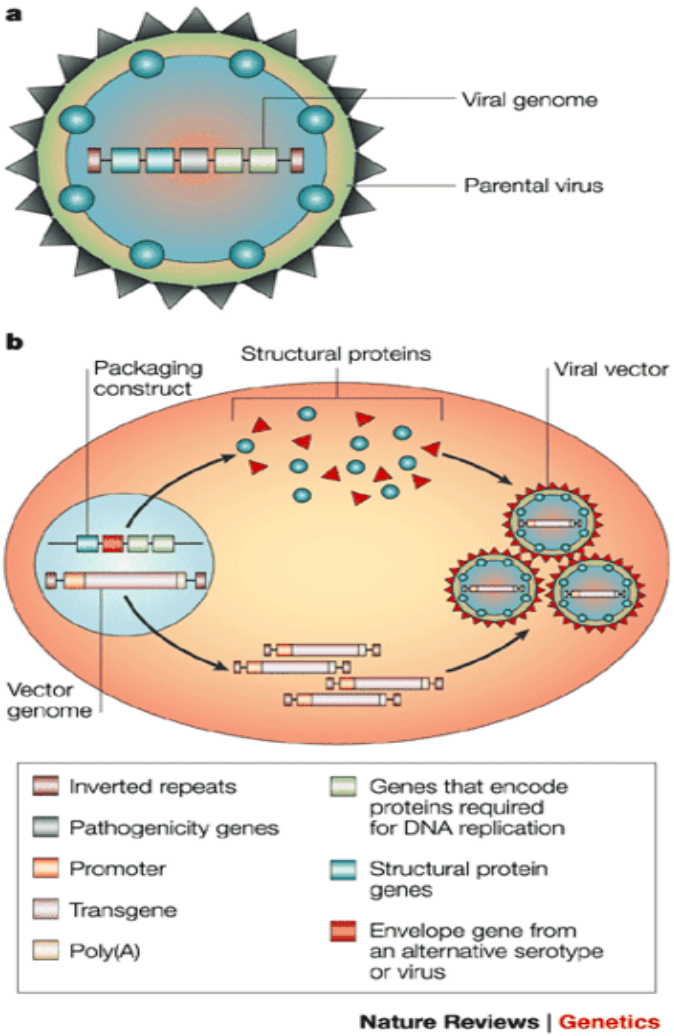
- Complete virus particles are released from the cell according to **vector-specific mechanisms**.

Production of gene transfer vector



Viral vector for gene transfer(حامل فيروسى لنقل الجين العلاجى)

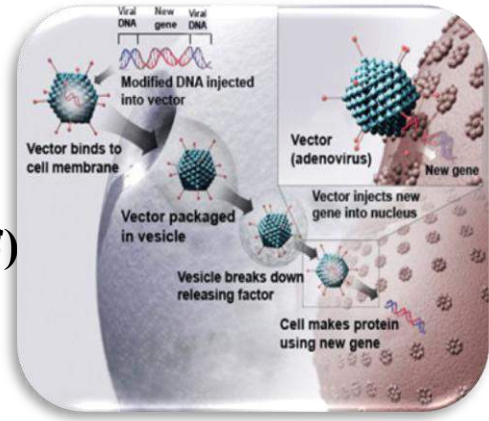
- **Viruses**, natural parasites that efficiently enter cellular targets and hijack cellular machinery for propagation.
- **Most effective** vectors for gene therapy (تعتبر من الحوامل الأكثر فعالية للمعالجة الجينية).
- Approximately **70%** of all gene therapy clinical trials employ **viral vectors.**
- To construct **viral vector** , the gene responsible for the virus replication and pathogenicity **removed** and replaced with **transgene cassette** **(يتم حذف الجينات المسؤولة عن تكاثر و فوعة الفيروس واستبدالها بالجين العلاجي)** .



Vectors currently in clinical use

Vector	Gene therapy clinical trials	
	Number	Percentage
Adenovirus	322	26
Retrovirus	293	23
Plasmid DNA	230	18
Lipofection	99	7.9
Vaccinia virus	88	7.0
Poxvirus	85	6.8
Adeno-associated virus	46	3.7
Herpes simplex virus	43	3.4
RNA transfer	16	1.3
Others[a]	31	2.4
Unknown[a]	36	2.9

Suitability (ملائمة)of adenovirus for gene transfer(لنقل الجين)



- **Transgene expression (التعبير عن الجين المنقول)**from these vectors is **rapid (سريع)**and robust (قوي)and is **enhanced** with strong heterologous **promoters.**
- **physically stable (ثابت فيزيائيا).**
- Adenoviruses **do not integrate** into the **host genome(لايندمج مع جين المضيف)**

- **<u>host response occurs in three phases (استجابة المضيف تحدث في ثلاثة اطوار):</u>**

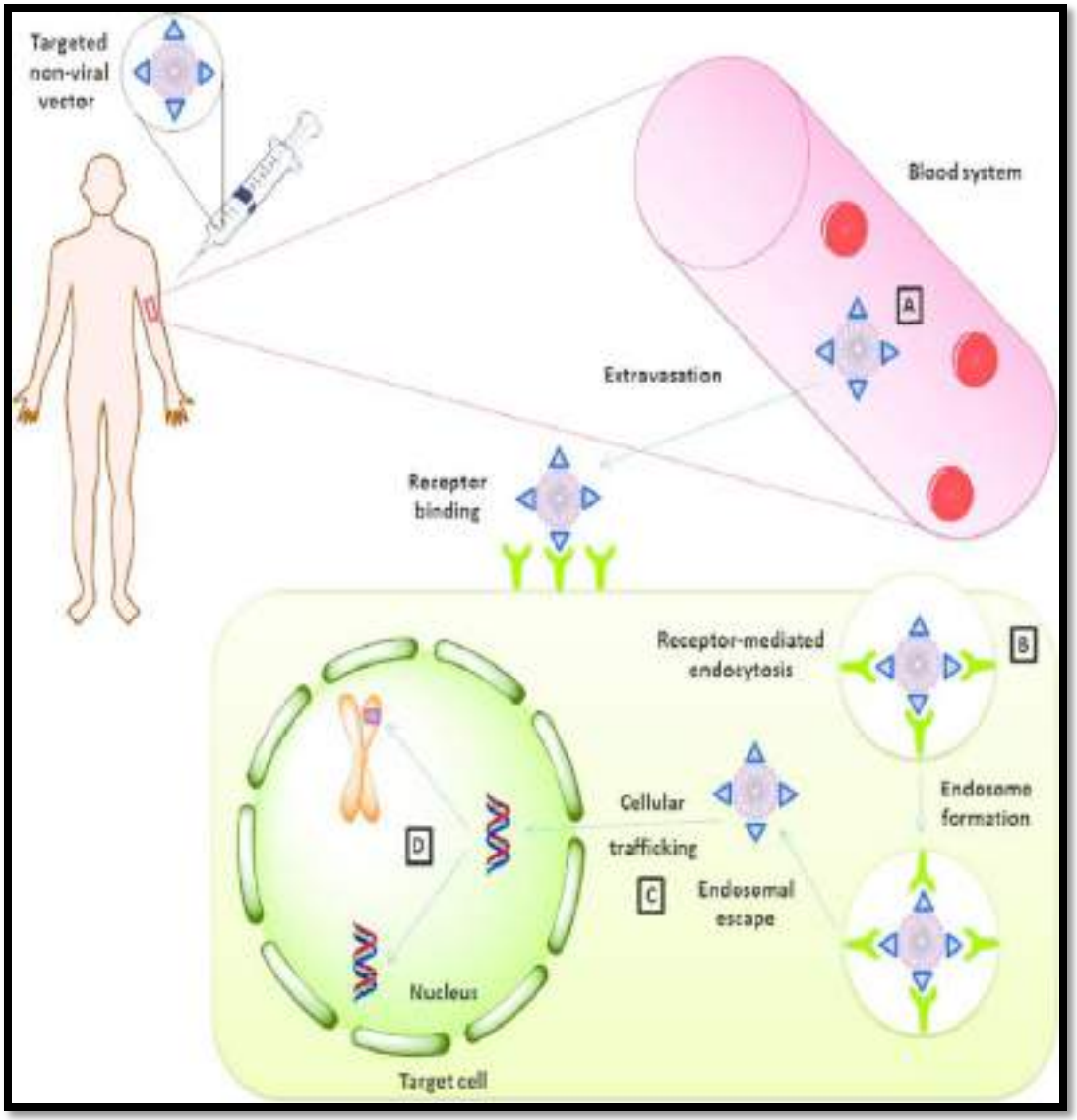
o **Phase** occurs within an hour after systemic administration خلال ساعة من) (الإعطاء الجهازي, lasts for 4 days and is characterized by thrombocytopenia (قلة الصفيحات)and elevated liver enzymes (dose dependent).

o **The second phase**, occurring 5 to 7 days after administration, is highlighted by removal of transduced cells (الخلايا التي نقل لها الحامل الجيني)by activated lymphocytes (اللمفاويات المفعلة)in the target tissue and localized, self-limited inflammation.

o **Third phase,** CD4 + T-cell-dependent humoral immunity مناعة) (خلطيةdevelops and neutralizing antibodies clear the virus from the circulation and prevent effective readministration.

Non viral vector (حوامل غير فيروسية)

- **Non-viral** vectors generally consist of **double-stranded** recombinant **DNA** plasmids **alone** or **encapsulated** (متمحفظة)in cationic **polymer** (بوليميرات موجبة الشحنة)or **lipid-based formulations**(تراكيب لبيدية).



Non viral vector

- **Non-viral vectors** offer several important **advantages (ميزات)** over **virus-based methods** for gene transfer:

  - Unlimited **cloning capacity (استطاعة تنسيل عير محدودة و عالية)** .

  - **non-immunogenic (غير مولدة للمناعة)** and can easily be **readministered multiple times** without induction of a **prohibitive immune** response (استجابة مناعية تثبيطية).

  - reduced capacity for **insertional mutagenesis (اقل قدرة على توليد الطفرات الناتجة عن الإدخال)** and a limited ability to produce unwanted **by-products** in vivo due to homologous recombination.

  - **Easy to manipulate** using standard techniques.

  - **Inexpensive** to produce, especially on a large scale in **contrast to viral vectors**.

Non viral vector

- **Have some disadvantages (تملك بعض السيئات) :**

- **Low transduction efficiency** non-specific uptake of the vector (القبط الغير نوعي للحامل) and **poor delivery** to the therapeutic target (تسليم بطيئ للهدف العلاجي) .

- limited capacity to **override cellular gene silencing** mechanisms (تجاوز اليات التثبيط الجيني)and, as a result, **cannot achieve sustained(مستمر) gene expression**.

Delivery methods for non viral gene transfer (طرق ايصال الجين الغير فيروسية)

- **Naked DNA** is susceptible to **nuclease degradation (تحطم)** in the systemic circulation (في الدوران الجهازي)and is taken up in **an inefficient, non-specific manner** in many tissues.

- **Physical methods (الطرق الفيزيائية)**used for gene transfer involve **disruption** of cell **membranes**.

- **Chemical methods (الطرق الكيميائية)**facilitate **interaction** with tissue targets and **transport** across cell membranes.

Physical method for gene transfer

- The **primary techniques (الطرق الأولية)**for recombinant DNA delivering to the target cells are **Microinjection (الحقن المجهري), particle bombardment (تفجير الجسيمات)**and **electroporation.**

- **Microinjection**: direct injection of DNA or RNA into the **cytoplasm** or **nucleus** of a single cell.

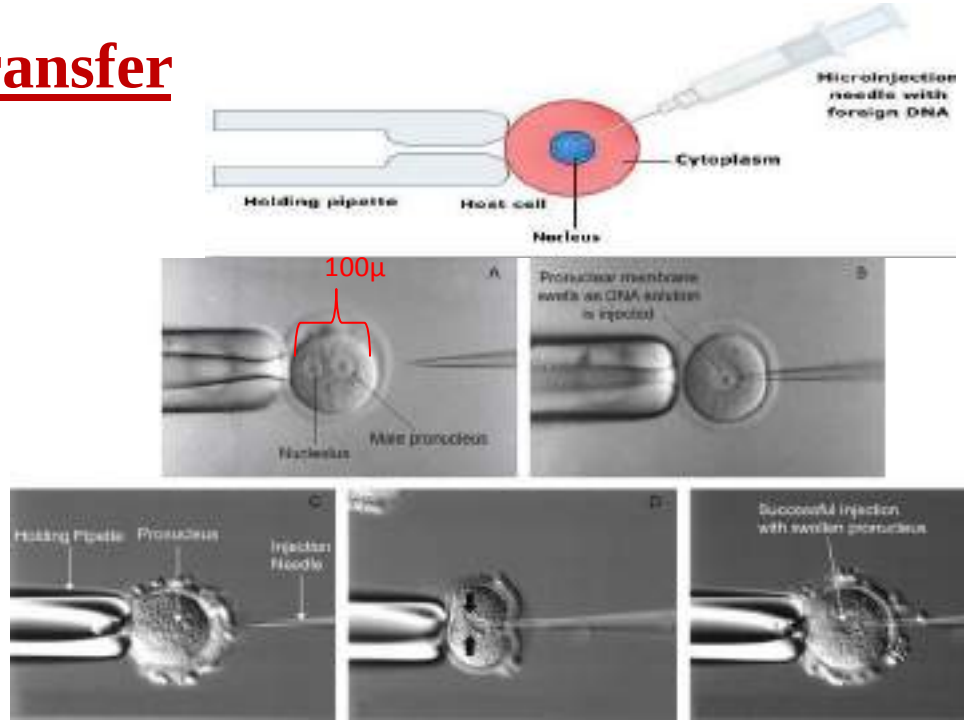
  - **simplest** and **most effective (الأكثر فعالية)**method for physical delivery of genetic material to cells.

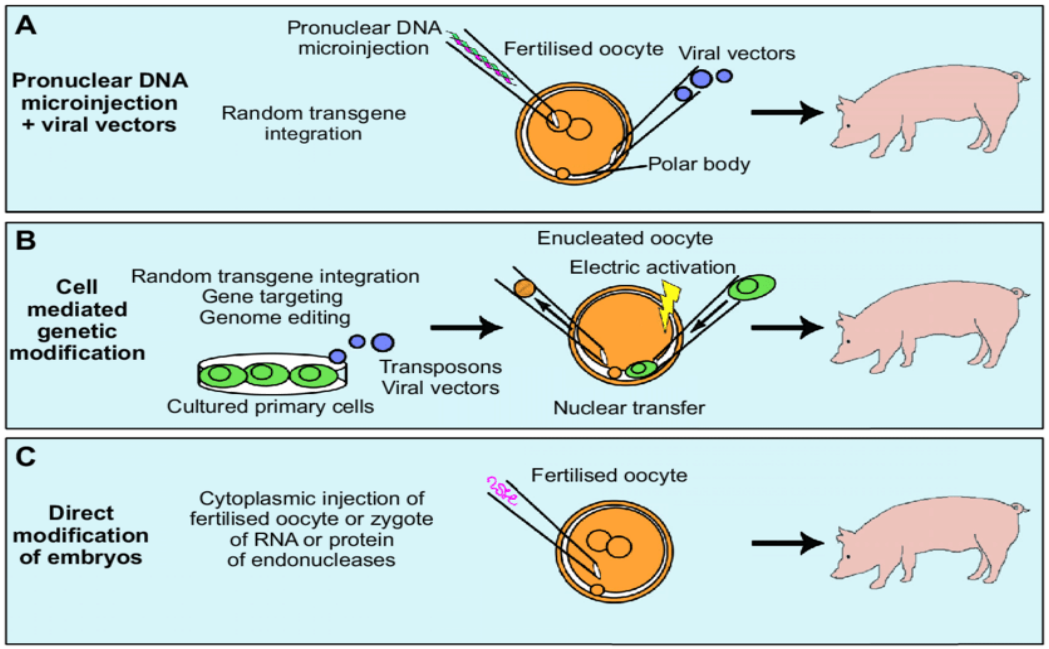
  - **transduces 100%** of the recipient cells (جميع الخلايا المستقبلة) and minimizes waste of plasmid DNA.

  - **But,** requires highly specialized equipment and skills.

  - **harsh to the cell.**

  - restricted to **ex vivo gene (خارج جسم الكائن الحي)** transfer of **cultured cells or embryonic stem cells** for production of transgenic animals.





Physical method for gene transfer

- **Particle bombardment (Gene gunبندقية الجين):**

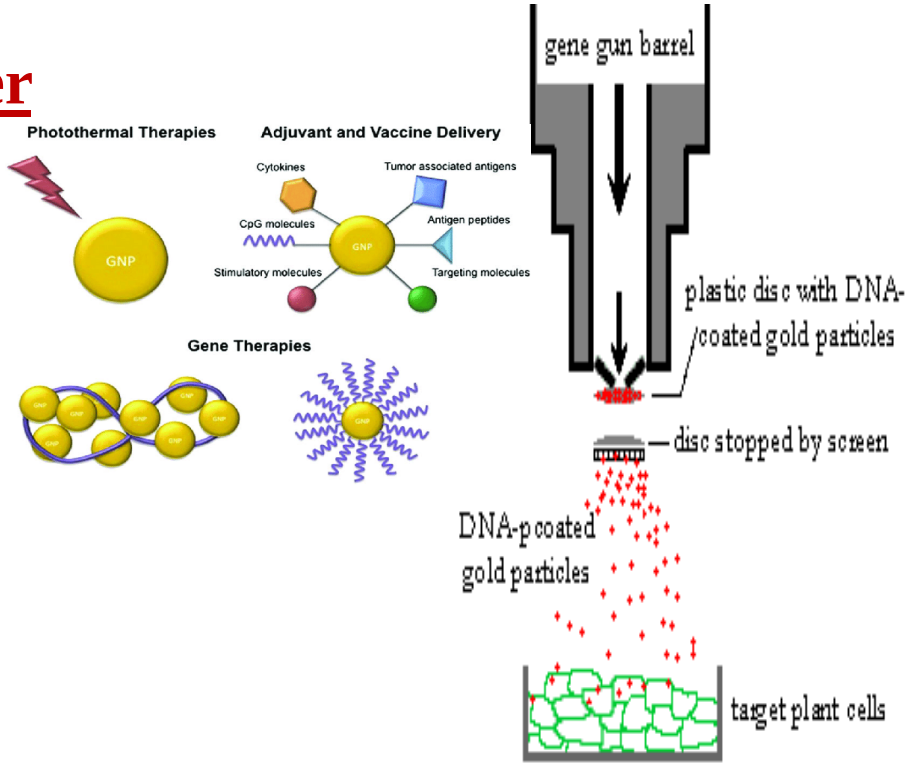
o **Gold particles (جسيمات من الذهب)**are coated with recombinant DNA and propelled (يتم دفعها)by an electric spark (شرارة مهربائية)or helium discharge into the target cell or tissue (Accell , helios gene gun devices).

o Transduction efficiency and distribution of gene expression relies upon **particle size**, **timing of delivery (الزمن)**and **particle acceleration (تسارع الجسيمات)**.

o This method gives **high levels** of transgene expression with very **low doses of DNA(كمية قليلة)**.

o But has **limited depth** of penetration(عمق نفوذ قليل).

o cells that directly encounter DNA-coated particles can be **severely damaged (تخرب شديد للخلايا)** .



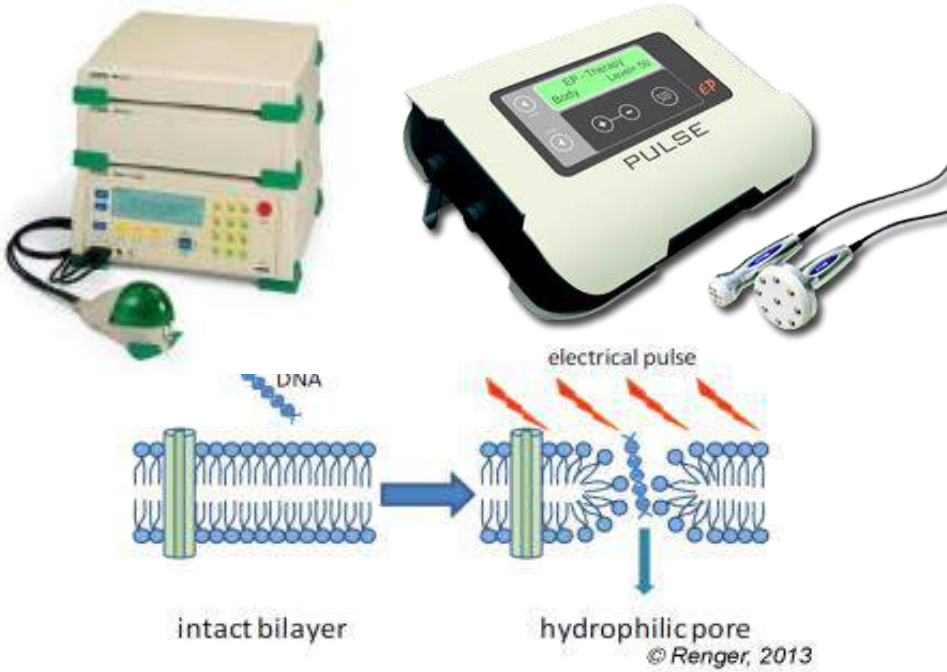
Gene gun Helics™ by BioRad is used to transfect cells in cultures and plant leaves

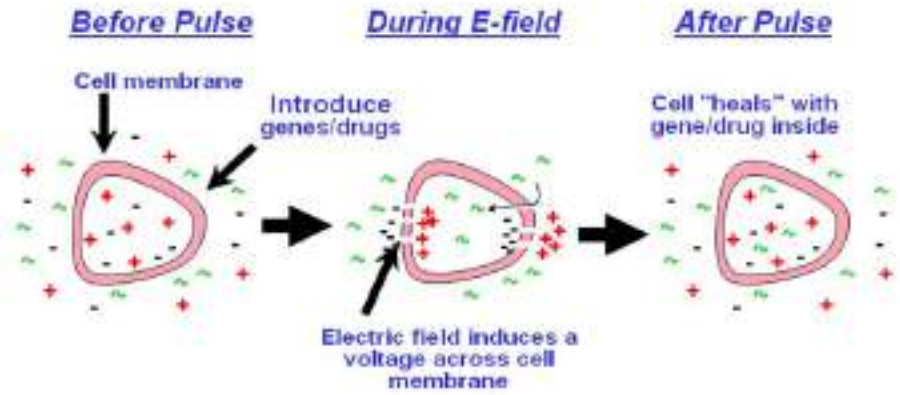
Physical method for gene transfer

- **Electroporation :**

o exposes the cell membrane to high-intensity pulses of electricity (نبضات كهربائية)that transiently destabilize the cell membrane and make it highly permeable to plasmid DNA.

o Had success in muscle, skin, liver and solid tumors.



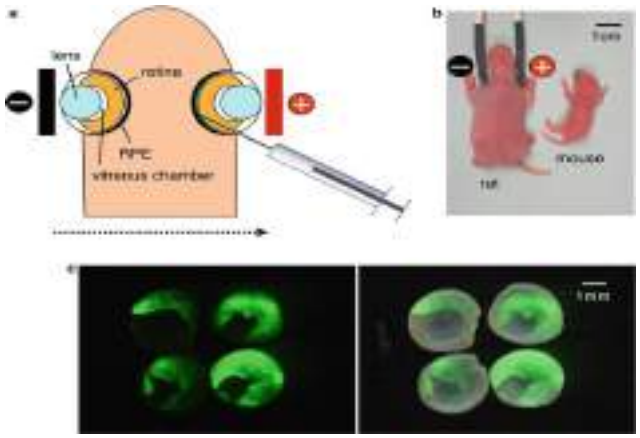


Physical method for gene transfer

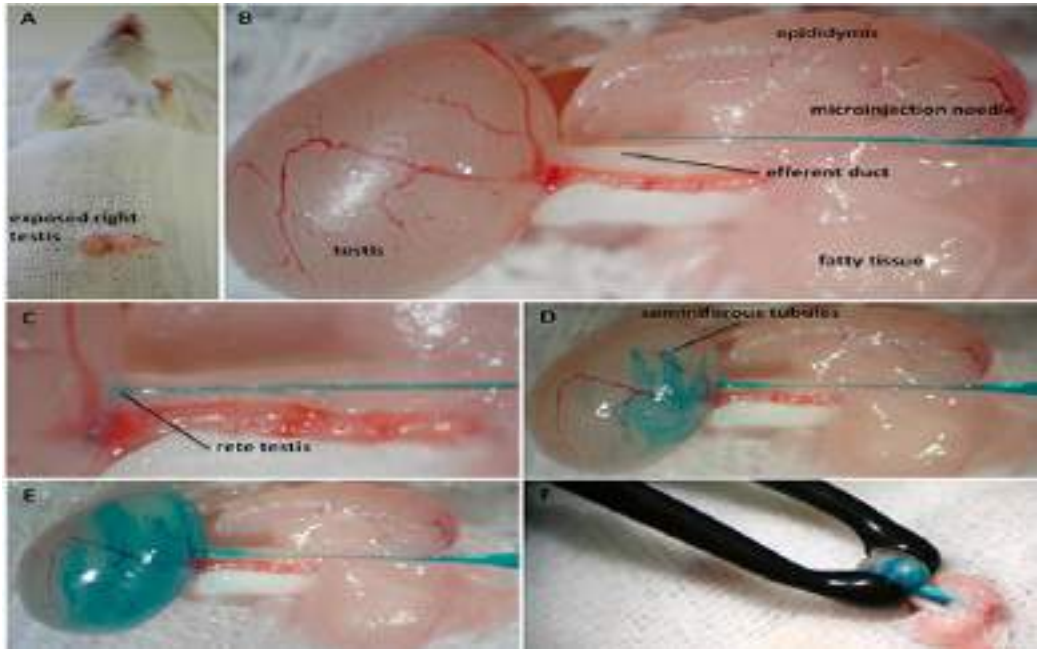
- **Electroporation :**

o consist of a **pulse generator** (مولد نبضات) **and** an applicator with several specialized electrodes tailored for delivery to specific tissues and organs.

o The parameters of **pulse duration** (مدة النبضات) and **field strength** (شدة الحقل الكهربائي) also **DNA concentration**(تركيز الدنا) have to be set depending on the cell type and the DNA molecule size for **efficient electroporation.**



***In vivo* electroporation**
(داخل جسم الكائن الحي)



Physical method for gene transfer

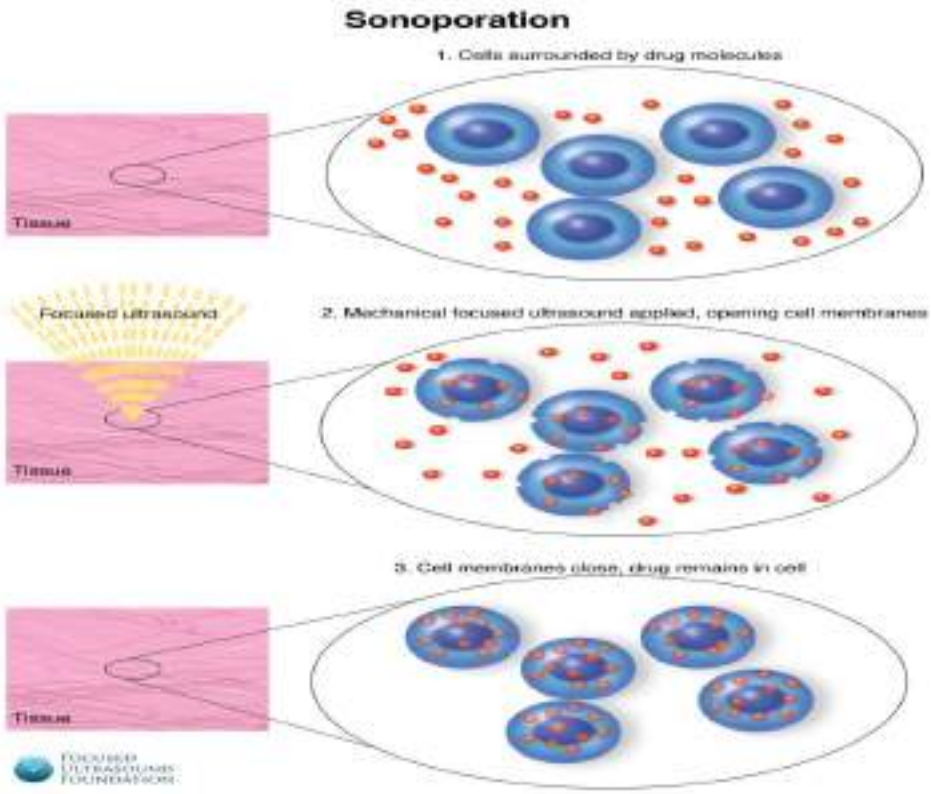
- **Sonoporation (معالجة الخلايا بالأمواج فوق الصوتية أو صوتنة الخلايا) :**

o Last 10 years new **less invasive method (طريقة غير باضعة).**

o Enhances cell membrane permeability by acoustic cavitations (التجاويف المحرضة بفعال الأمواج فوق الصوتية) through **ultrasound waves collapse** active bubbles, releasing **energy** that disrupts **adjacent cell membranes.**

o Have been applied ***in vitro*** and ***in vivo***.

o Have a good **safety profile** in clinic.



Physical method for gene transfer

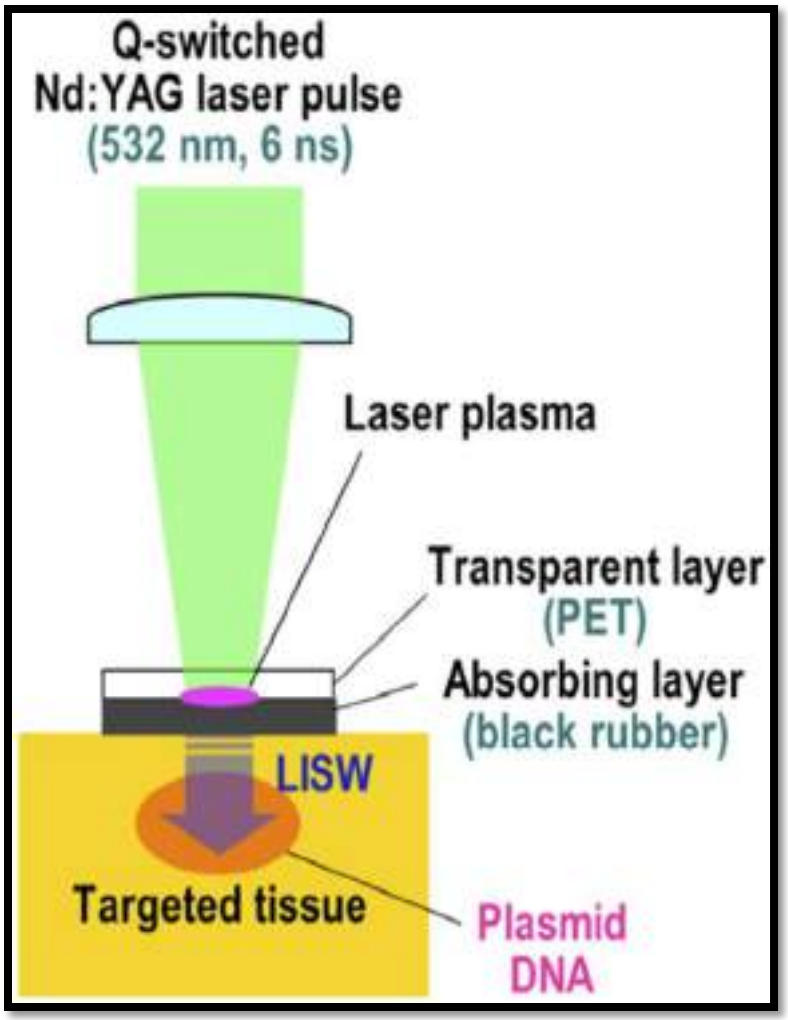
- **Laser irradiation (التشعيع بالايزر):**

- focusing a **laser beam** on a target cell and modifying permeability by **thermal effects (تأثيرات حرارية).**

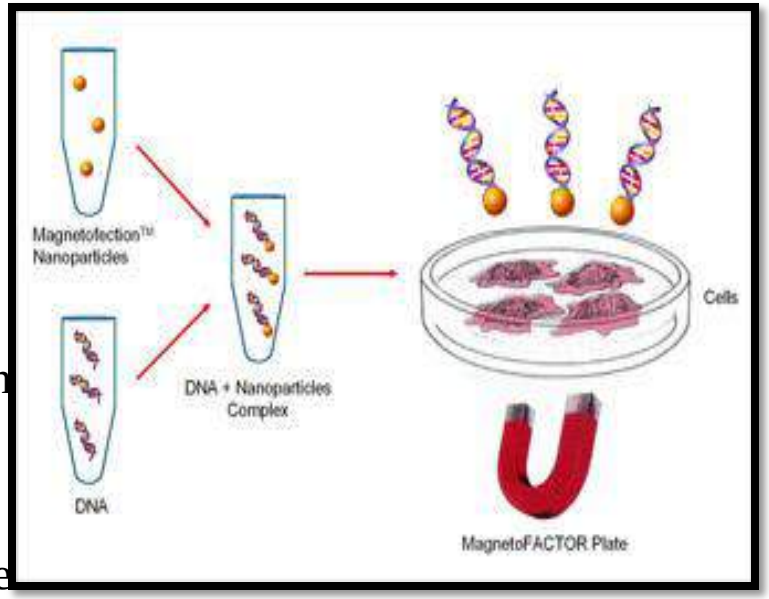
- Laser irradiation induces **minimal cell damage because permeabilization is transient** and very **fast (عابر و سريع)**.

- **100% transduction** efficiency without affecting cell **growth** and **division**.

- The **size** and **expense** (الكلفة و الحجم الكبير)of the laser source limited the use of this technique.



Physical method for gene transfer



- **Magnetofection (معالجة الخلايا بتطبيق حقل مغناطيسي):**

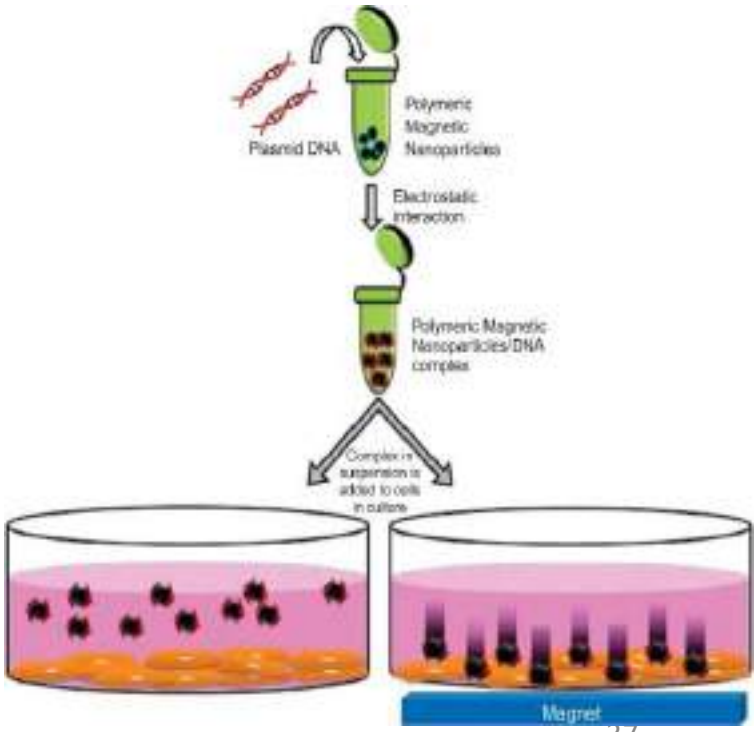
o Involves attachment of **magnetic polymer-coated** iron oxide-nanoparticles to DNA (ربط جسيمات نانوية مغناطيسية من اوكسيد الحديد الى الدنا).

o **Magnetic particles (الجسيمات المغناطيسية)**are concentrated in **target cells** by an external magnetic field (حقل مغناطيسي)that pulls the particles across **plasma membranes** into the **cytoplasm.**

o High **transduction efficiencies** have been achieved with this method **in vivo in the gastrointestinal** tract and **blood vessels (الطريق المعدي المعوي و الأوعية الدموية)** .

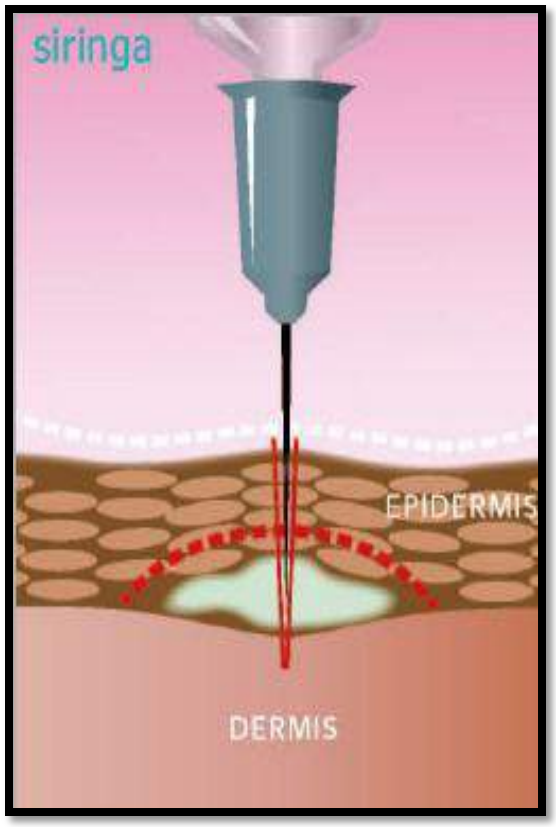
o Vector type (viral vs. non-viral), dose, composition and incubation time influence transduction.

o **advantage** of magnetofection is that it increases **bioavailability (زيادة التوافر الحيوي للدنا المؤشب)**of recombinant DNA and reduces the amount needed for effective gene transfer(تقلل من كمية الدنا اللازمة)



Physical method for gene transfer

- **Hydroporation (نقل الدنا في محلول مائي و تشكيل الثقوب):**



- Involves **injection of large volumes** of solution into the **circulation** (حقن كمية كبيرة من محلول حاوي على الدنا الى الدوران) **to overcome the physical barriers** of the endothelium and the cell membrane This technique requires only a **needle and syringe,** Ringers solution and phosphate buffered saline have also been employed.

- The **dose** of DNA (جرعة الدنا) delivered by hydrodynamic delivery ranges from **0.1 to 10 mg/kg.**

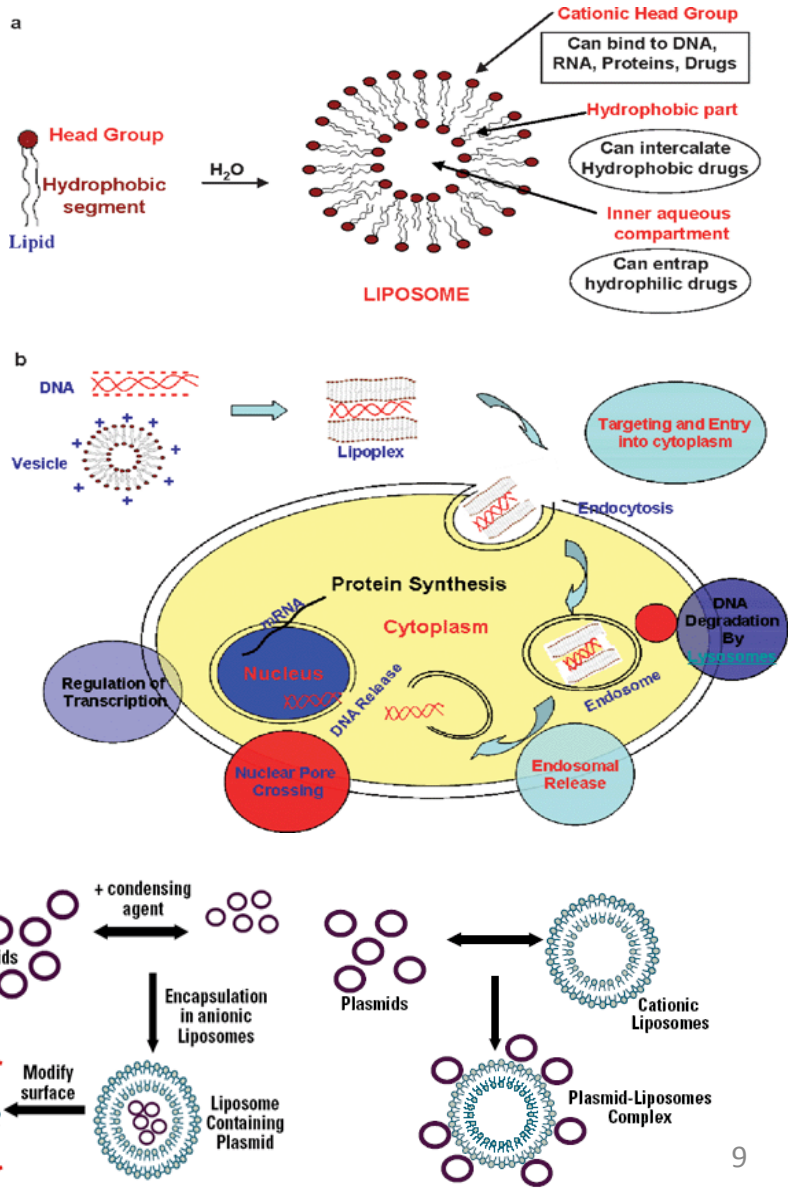
- This technique could increase the **blood pressure** and **decrease the heart rate**.

- Increase in **transaminase** after liver injection and **creatinine kinase** after muscle injection (الحقن العضلي) .

Chemical methods for gene transfer

- **Cationic liposoms (الليبوزومات موجبة الشحنة):**

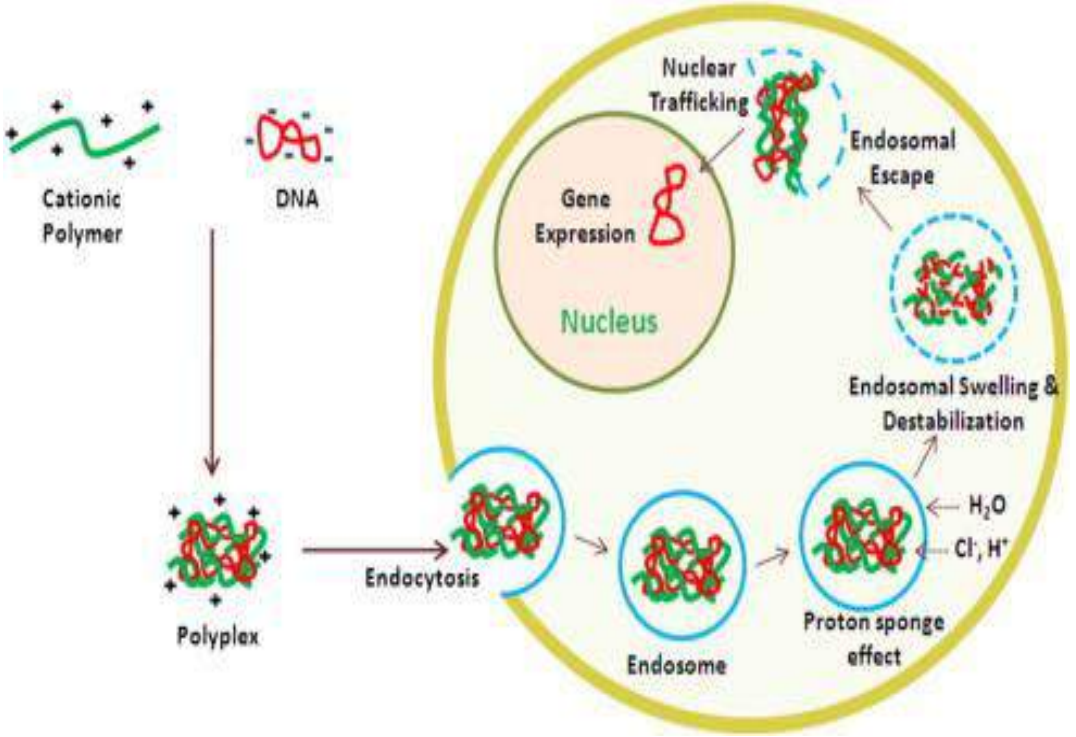
  - **Liposomal** gene delivery was the first **non-viral system** to reach clinical trials.
  - There is natural interaction (تداخل طبيعي) of **cationic liposomes** with the **negatively** charged phosphate backbone of recombinant **DNA** to form organized structures **protect** the **genetic material** from degradation.
  - **The positive charge (الشحنة الإيجابية)** also promoted interaction with the **cell membrane** and endocytosis (الإلتقام).
  - **Interaction DNA-liposome** , depend on the **PH, charge** and **lipid structure**.



Chemical methods for gene transfer

- **Cationic polymer (بوليميرات موجبة الشحنة):**

- **Cationic polymers** condense DNA by **neutralizing the charge of the DNA** backbone and mediate cellular contact through **ionic interaction.**
- **Polylysine** (PLL) and **polyethylenimine** (PEI) are the most commonly used cationic polymers.
- Covalently bind (ترتبط بشكل تشاركي)to compounds that interact with specific **cell surface marker.**
- Other biodegradable polymers such as poly(a-[4-aminobutyl]- L-glycolic acid **(PAGA)** have **reduced toxicity** associated with DNA polyplexes and **improved gene expression (انقصت من السمية و حسنت من التعبير الجيني).**



Immune response against non viral vector (الإستجابة المناعية ضد حامل الجين الغير فيروسي)

- There is a **little effect** with **the low dose (جرعات منحفضة)** with non viral DNA complexes.

- **Higher doses (الجرعات العالية)**, especially of **cationic liposomes**, induce **acute inflammation and profound tissue damage (التهاب حاد و تخرب بالأنسجة)** .

- The most **severe side effects (معظم التأثيرات الجانبية الشديدة)** occur after **intravenous and intrapulmonary delivery.**

Immune response against non viral vector

- **The inflammation and the toxicity of the non viral vector can be reduced by:**

o Removal **of CpG motifs (نزع التسلسل المتكرر)**in plasmid DNA, minimizing interaction (يقلل التداخل) between the complex and the **immune system** and use of immunosuppressants in the complex.

o Covalent attachment (الربط التشاركي)of **poly(ethylene) glycol** to the **surface of DNA** complexes (PEGylation) has improved toxicity and promotes transduction efficiency by preventing aggregation (تقلل السمية و تحسن من فعالية النقل).

o Injection of **lipids (حقن لبيدات)prior** of administration of recombinant DNA **reduced cytokine production by 80%** in mice by changing the **tissue distribuation (عن طريق تغير التوزع النسيجي)**.

Chemical methods for gene transfer

	Advantages	Disadvantages
Naked DNA	No special skills needed Easy to produce	Low transduction efficiency Transient gene expression
Physical methods		
Microinjection	Up to 100% transduction efficiency (nuclear injection)	Requires highly specialized skills for delivery Limited to ex vivo delivery
Gene gun	Easy to perform Effective immunization with low amount of DNA	Poor tissue penetration
Electroporation	High transduction efficiency	Transient gene expression Toxicity, tissue damage Highly invasive
Sonoporation	Method well tolerated for other applications	Transient gene expression Toxicity not yet established
Laser irradiation	Can achieve 100% transduction efficiency	Special skills and expensive equipment necessar
Magentofection	Safety of method established in the clinic	Poor efficiency with naked DNA
Chemical methods		
Liposomes	Easy to produce Fusion liposomes improve transduction efficiency	Transient gene expression Toxicity, mildly immunogenic
Cationic polymers	Easy to manipulate for targeting	Transient gene expression Toxicity, mildly immunogenic

Clinical used of non viral vectors (الإستخدام السريري للحوامل الغير فيروسية)

- **Vaccination (التلقيح)** against **HIV-1** and are in phase I testing.

- Three **plasmid DNA vaccine** for Ebola (لقاح ضد الإيبولا)infection has completed **phase I testing** and will **enter phase II alone** and in combination with a **recombinant adenovirus** vector in a prime-boost dosing regimen.

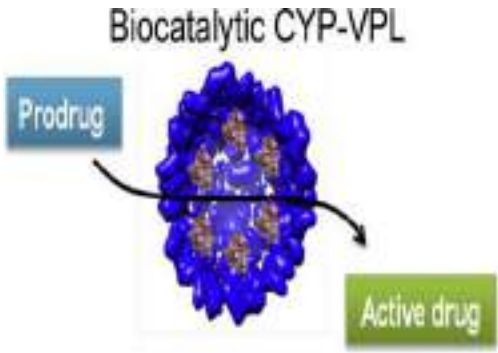
- human trials using **non-viral vectors** to treat genetic diseases such as **hemophilia A** , disease illustrate the challenges of permanent correction of a hereditary disorder with a plasmid-based system (اضطراب وراثي باستخدام نظام معتمد على البلاسميد العلاجي)

The role of Drug metabolism in gene therapy (دور استقلاب الدواء في المعالجة الجينية)

- ***In vitro***, ***in vivo*** and clinical observations have documented that infection and inflammation (الخمج و الإلتهاب)significantly reduces the expression and function of **cytochrome P450** (CYP) enzymes.

- Single dose of **recombinant adenovirus suppresses** rat **CYP3A2** for 14 days without resolution, CYP3A2 is homologous (مماثل)to human **CYP3A4**, responsible for the metabolism of approximately **50% of marketed medications**.

- Understanding the effects of **viral and non-viral vectors** on **CYP** and other **drug metabolizing enzymes** is important since traditional **drug** regimens are also **included** in many **gene therapy** trials.



Production and processing of non viral vector

- **Fermentation (التخمر والزرع) .**
- **Harvest (جمع الناتج).**
- **Lysis (حل الخلايا).**
- **Isolation and purification (العزل و التنقية).**
- **Bulk preparation (تحضير الكمية و الحجم).**

Quality control (ضبط الجودة)and acceptable levels of impurities (الشوائب) in the final plasmid - based product

type	Issue	Determined by	Acceptable level in final product
Identity	Cross-contamination with other products	Restriction digest/gel electrophoresis	N/A
Purity	Residual bacterial chromosomal DNA	Real-time PCR	<2 μg/mg pDNA
	Residual RNA	Analytical HPLC	<0.2 μg/mg pDNA
	Residual bacterial protein	BCA protein assay	<3 μg/mg pDNA
	Endotoxin	LAL assay	<10 E.U./mg pDNA
	Sterility (bacterial and fungal)	Method outlined in CFR 21 610.12	No growth
	Appearance	Visual inspection	Clear solution free of particulates
	pH	pH meter	Physiologic (7.0–7.4) but may be product specific