TOPIC: RECOMBINANT DNA TECHNOLOGY APPLICATIONS – PART III

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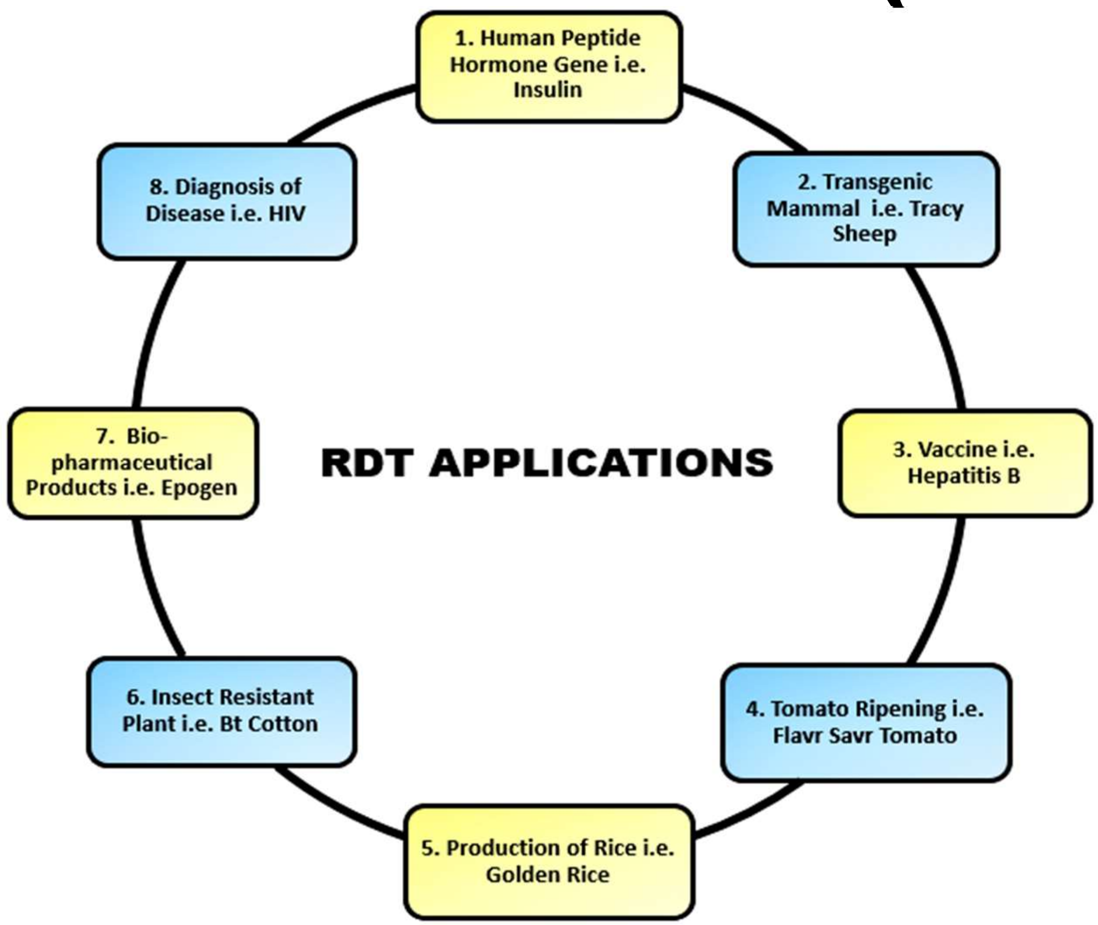
APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY (RDT)



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APPLICATIONS OF RDT IN FIELD OF AGRICULTURE

1. NUTRITIONAL ENHANCEMENT OF CROP

(A) PRODUCTION OF GOLDEN RICE

- **Vitamin A deficiency is prevalent in many areas of Asia and Africa, and more than 500,000 children a year become permanently blind as a result of this deficiency.Rice is a major staple food in these regions but does not contain vitamin A.**
- **Many children in countries where rice is a dietary staple loose their eyesight because of diets deficient in vitamin A. Golden rice, a strain genetically modified to produce β-carotene , is a precursor to vitamin A.**
- **White rice lacks the enzyme phytoene synthase, which is responsible for converting C20 into phytoene, a rate- limiting step in the production of β-carotene. Introducing the phytoene synthase gene into rice is one of the way to overcome this block and produce golden rice enriched in β-carotene.**
- **To create golden rice ,scientists transferred into rice genome by RDT three genes encoding enzymes required for the biosynthetic pathway leading to β-carotene synthesis—**
  (i) Phytoene synthase (*psy,* from daffodil)
  (ii) Phytoene desaturase (*crt*I from bacteria)
  (iii) Lycopene beta-cyclase *(lcy,* from daffodil)

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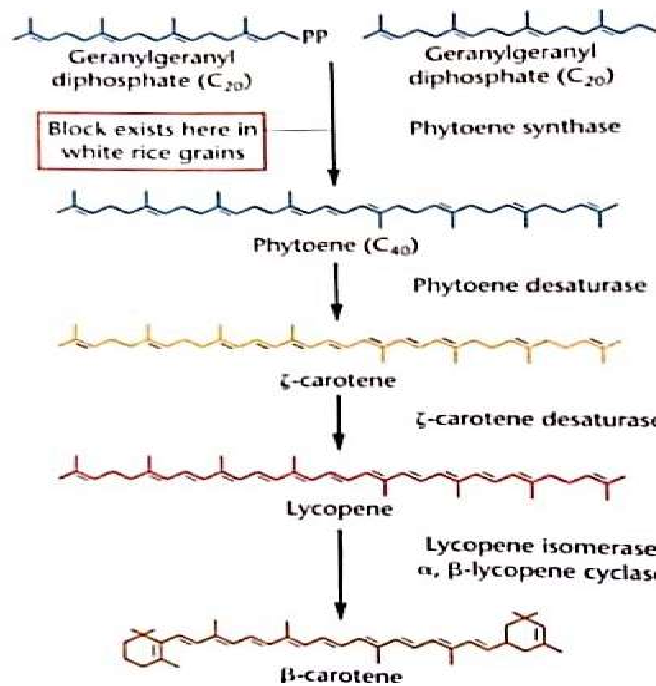
- Golden Rice 1 was developed to help control vitamin A deficiency (VAD). To construct Golden Rice 2, the phytoene synthetase gene (*psy*) from maize and the carotene desaturase gene (*crtl*) from *Erwinia uredovora* were inserted into rice.

- Golden Rice 1 contains about 1.6 g of total carotenoids per gram of dry weight of grain. Golden Rice 2 contains as much as 37 g total carotenoids per gram of dry weight of grain, of which 31 g/g is -carotene (20 times more). While the quantity of -carotene is high, its bioavailability is unknown. Golden Rice 2 was developed with the expectation that it could make a major contribution to the vitamin A requirement.



B-CAROTENE PATHWAY –THE GOLDEN RICE SOLUTION

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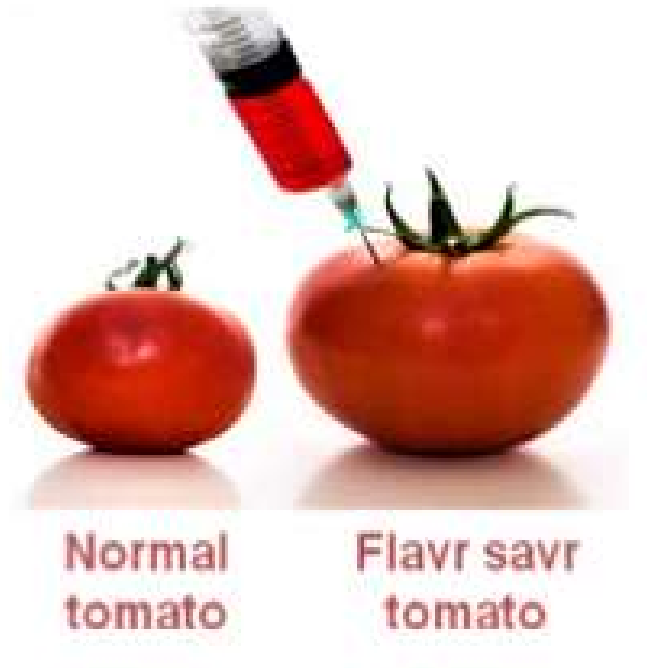
(B) PRODUCTION OF SLOW RIPENING FRUITS

- **Among this the first genetically modified food licensed for human consumption was Flavr Savr tomato.**
- **The ripening process in these tomatoes is slowed down by inhibition of an enzyme polygalacturonase which resulted in delayed ripening and resistant to rotting.**



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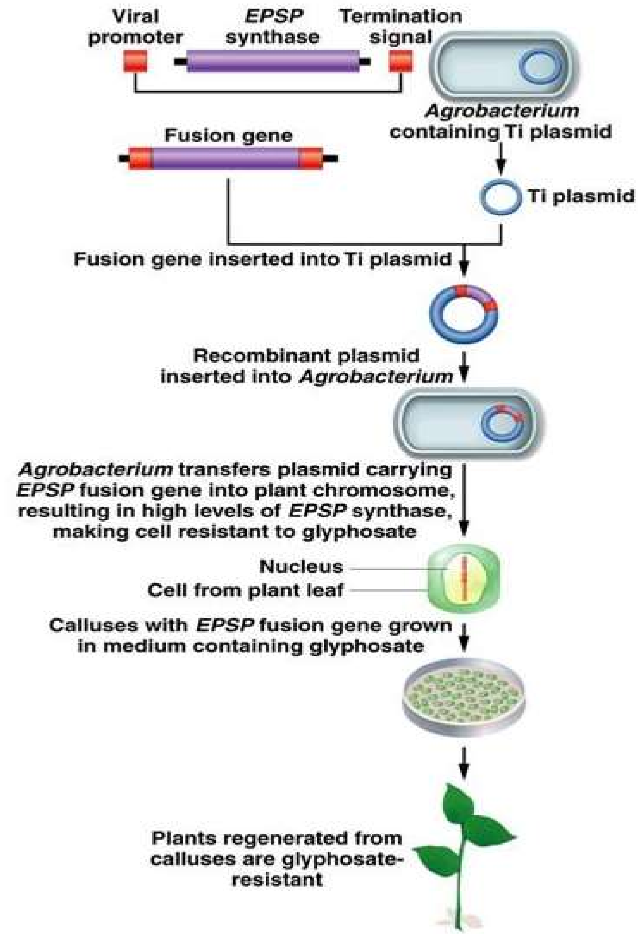
2. RESISTANCE TO HERBICIDE & PEST

(A) HERBICIDE-RESISTANT TRANSGENIC PLANT

- The creation of herbicide-resistant plant has opened the way to efficient weed control and increased yields of major agriculture crops.
- One of the herbicide-resistant plant is Glyphosate Herbicide-resistant Plant.
- To create glyphosate-resistant transgenic plants, the EPSP synthase gene from bacteria is fused to a promoter such as the promoter from the cauliflower mosaic virus. This fusion gene is then ligated into a Ti -plasmid vector, and the recombinant vector is transformed into *R.radiobactier* host cells*. R.radiobactier infection* of cultured plant cells transfers the EPSP synthase fusion gene into a plant cells chromosome. Cells that acquire the gene are able to synthesize large quantities of EPSP synthase making them resistant to the herbicide glyphosate. Resistance cells are selected by growth in herbicide containing medium. Plant generated from these cells are herbicide resistant.



OUTLINE FOR FORMATION OF GLYPHOSATE RESISTANT PLANT

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(B) INSECT-RESISTANT TRANSGENIC PLANT

- *Bacillus thuringiensis* (Bt) is a gram positive soil bacteria that produces crystalline toxins on sporulation coded by cry genes.
- To produce insect-resistant transgenic plant, the DNA sequence coding for the toxin is removed from the bacterium and with suitable promoter and terminal sequences recombinant DNA is constructed and introduced into plant using *Agrobacterium tumefaciens* as vector.
- The Bt toxin encoded by the genes cry I Ac and cry II Ab control bollworms while cry I Ab toxin control corn borer.
- Bt toxins are produced in inactive form but converted to its active form in the alkaline pH of insect gut.
- Bt toxins bind to the inside of the insect gut and damage its epithelium so that it cannot absorb digested food and starved to death.
- Transgenic cotton, tomato, potato and tobacco plant resistant to various pest have been transformed.

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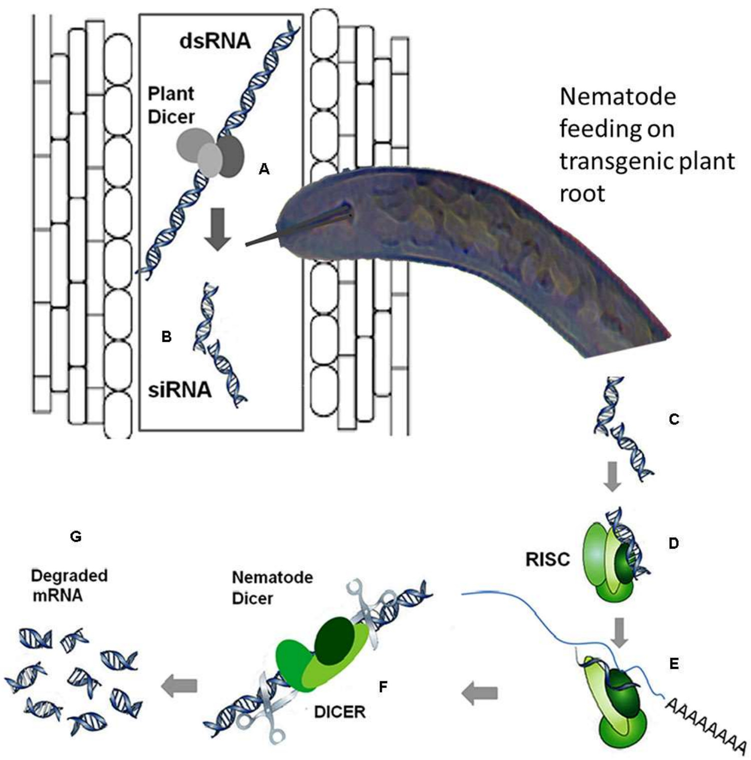
(C) NEMATODE-RESISTANT TRANSGENIC PLANT

- The nematode *Meloidogyne incognita* infects the roots of tobacco and rice plants, causing great reduction in yield.

- To prevent infection, genes of nematode involved in parasitism were introduced into the host plant by Agrobacterium Vector, leading to production of both sense and antisense RNAs in the host cells.

- The strategy, called RNA interference (RNAi), silence the specific RNA of the parasite by preventing its translation through ds RNA formation.



Overview of Strategy – RNA interference (RNAi) in Nematode Resistant Transgenic Plant

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LIST OF TRANSGENIC PLANTS

Crop	Genes transferred	Purpose
Tobacco	*B. Thuringiensis* *CpT1* Chitinase gene *aro* A Farnesyl diphosphate synthase gene of *S. cerevaisae*	Insect resistant Insect resistant Disease resistant Herbicide resistant Increased in sterol and carotenoid synthesis
Tomato	*Bt* *aro* A Sucrose phosphate synthase	Insect resistant Herbicide resistant Increased sucrose and reduced starch
Potato	*Bt* Bacteriaphase T-4 lysozyme Bacterial ADP-GPPase	Insect resistant Disease resistant Increased starch amino acid
Cotton	*Bt*	Insect resistant
Rice	*Bt*	Insect resistant
Wheat	CP4-EPSPS and *gox* High molecular glutenin gene	Herbicide resistant Starch content modified
Maize	*Bt*	Insect pest resistant
Sugarcane	*Bt*	Insect pest resistant
Soybean	CP4-EPSPS	Herbicide resistant
Sugarbeat	CP4-EPSPS	Herbicide resistant

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APPLICATIONS OF RDT IN FIELD OF MEDICINE

1. PRODUCTION OF INSULIN

- **The first human gene product manufactured by recombinant DNA technology was human insulin called Humulin, at Genentech (Genetic engineering technology),regarded as the world's first biotechnology company and licensed for therapeutic use in 1982 by the U.S. Food and Drug Administration (FDA)**

- **To synthesize recombinant human insulin, synthetic oligonucleotides encoding the insulin A and B chains (63 nucleotides for the A poly-peptide and 90 nucleotides for the B polypeptide) were inserted (in separate vectors) at tail of a cloned *E. coli lacZ* gene .The recombinants plasmids were transformed into *E.coli* host cells, where the β-gal/ insulin fusion protein was synthesized and accumulated in the cells. Fusion proteins were then extracted from host cells and purified. Insulin chains were released from β-galactosidase by treatment with cyanogen bromide. The insulin subunits were purified and mixed to produce a functional insulin molecule.**

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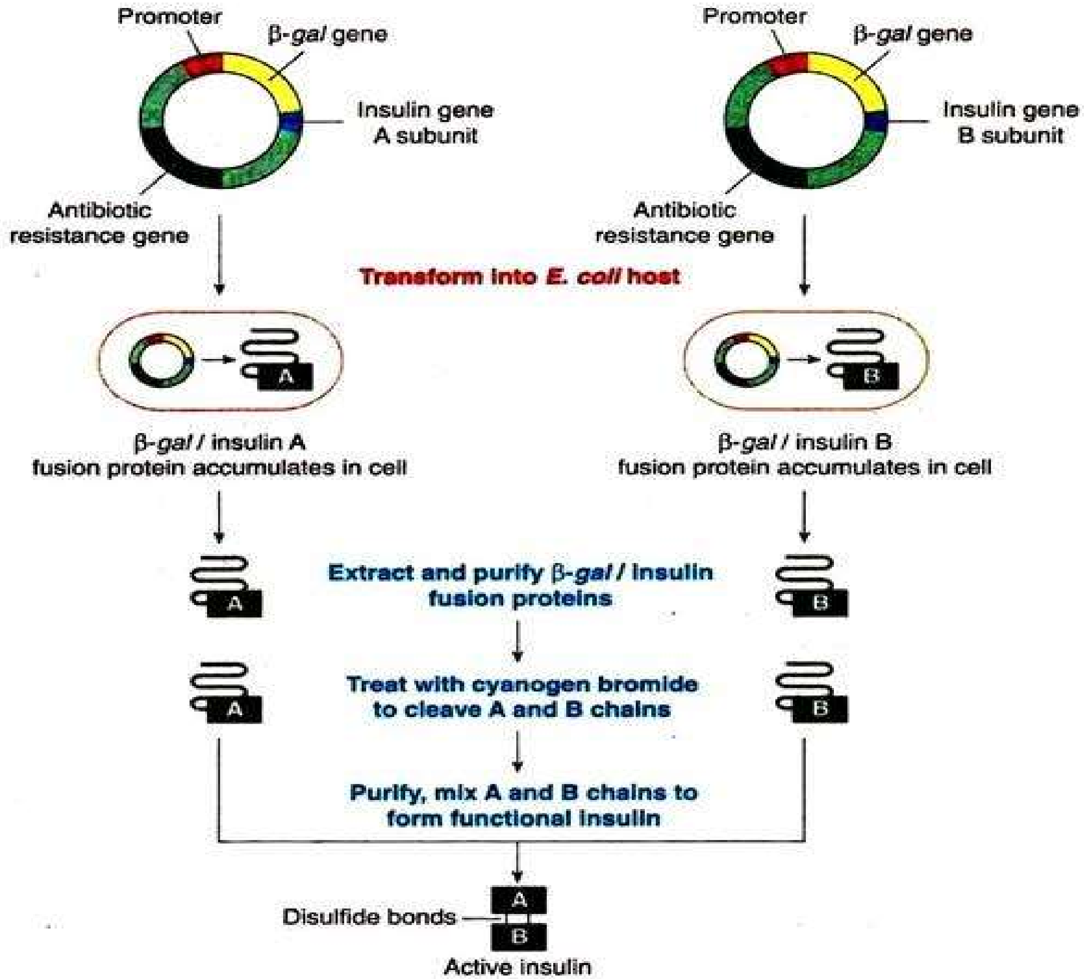

SYNTHESIS OF HUMULIN A RECOMBINANT FORM OF HUMAN INSULIN

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LIST OF IMPORTANT RECOMBINANT PRODUCTS SYNTHESIZED IN BACTERIA ,PLANTS ,YEAST & ANIMALS

Gene Product	Condition Treated	Host Type
Insulin	Diabetes	*E. coli*
hGH	Dwarfism	*E. coli* , cultured mammalian cell
Erythropoietin	Anemia	*E. coli*
Interferon	Sclerosis, cancer	*E. coli*
Tissue plasminogen Activator	Heart attack	Cultured mammalian cell
Monoclonal antibodies VEGF	Cancer	Cultured mammalian cell
Blood clotting factor VIII	Hemophilia A	Transgenic sheep, pig
Hepatis B surface protein vaccine	Hepatitis B	Cultured yeast cells, banana
Immunoglobin IgG1 to HSV-2	Herpes virus infection	Transgenic soybeans glycoprotein B
Recombinant monoclonal antibodies	Passive immunization against rabies, cancer, rheumatoid arthritis	Transgenic tobacco , soybeans, mammalian cells
Norwalk virus capsid	Norwalk virus infection	Potato edible vaccine
E.coli heat-labile enterotoxin	*E. coli* infection	Potato edible vaccine

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2. PRODUCTION OF SUBUNIT VACCINES

- A new type of vaccine by RDT consisting one or more surface proteins from virus or bacteria but not the entire virus or bacteria. Surface protein acts as an antigen to stimulate production of antibody.
- Hepatitis B virus vaccine - one of first subunit vaccine. The gene that encodes the hepatitis B surface protein has been cloned into a Yeast expression vector and the cloned gene is then expressed in yeast host cells. The protein is then extracted and purified from the host cells to be used as vaccine.
- In 2005, FDA approved Gardasil, a subunit vaccine produced by Merk as first cancer vaccine, which targets four strains of human papillomavirus (HPV) that causes cervix cancer. Approximately 70% of sexually active women may be infected by HPV. HPV is designed to provide immunity against HPV prior to infection (but not effective against existing infection)

3. PRODUCTION OF DNA VACCINES

- DNA encoding proteins from a particular pathogen are inserted into a plasmid vector and is then injected directly into an individual or delivered via a virus vector.
  e.g. Trials are under way to create vaccine against HIV/COVID-19
- Limitations: very low production of protein encoded by delivered gene.

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4. OTHER APPLICATIONS OF MEDICAL IMPORTANCE

A. In 1997, the first transgenic cow, ‘Rosie’ (and later Rosita) was developed which produced “human milk” (2.4 gm/liters) as its milk was enriched with human milk protein (α-lactalbumin), so it was more beneficial than normal cow milk.

B. The milk of transgenic sheep ‘Tracy’ contains a human blood protein (α-1-antitrypsin), used to treat ‘emphysema’- a lung disease if its gene mutates.

C. The transgenic sheep ‘Molly and Polly’ were created from adult somatic cells to obtain blood clotting protein ‘Factor IX’ whose absence causes ‘haemophilia B’.

D. The anti-blood clotting agent ‘hirudin’ is obtained from transgenic plant, *Brassico napus.*


Transgenic Cow - Rosie


Transgenic Sheep - Tracy


Transgenic Factor IX Sheep - Molly & Polly

Therefore, the most successful and widespread applications of RDT are in the production of recombinant proteins as bio-pharmaceutical products, the process is know as ‘Biopharming’.

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LIST OF SOME IMPORTANT PHARMACEUTICAL DRUGS PREPARED BY RDT & ITS APPLICATIONS

Drugs	Applications
Epogen/Procrit	For patients with anemia due to Dialysis/Chronic Kidney Disease/ Renal Failure/ Chemo / HIV
Neulasta	For Neutropenia; low WBC count febrile neutropenia (low WBC count with /infection) due to chemo, BMT,AML
Infergen	For patients with Chronic, non-responding, or relapsing hepatitis C viral (HCV) infection
Avonex	Treatment of relapsing forms of MS; slows the progression of MS by regulating the body's immune response against myelin.
Betaseron	Multiple Sclerosis; significantly delays the progression of secondary MS including relapsing-remitting MS
Forteo	Treatment of osteoporosis in women and men
Intron A	Treat different types of leukemia, malignant melanoma, multiple myeloma , basal cell carcinoma

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SIGNIFICANCE OF RDT APPLICATIONS

- RDT, genetic engineering, and biotechnology have revolutionized medicine and agriculture.
- Genetically modified plants and animals can serve as bioreactors to produce therapeutic proteins and other valuable protein products.
- Genetic modification of plants have resulted in herbicide-and pest-resistant crops, and crops with improved nutritional value; similarly , transgenic animals are being created to produce therapeutic proteins and to protect animals from disease.
- A synthetic genome has been assembled and transplanted into a donor bacterial strain elevating interest in potential applications of synthetic biology.
- Applications of recombinant DNA technology and genomics have become essential for diagnosing genetic disorders, determining genotypes ,and scanning the human genome to detect disease.
- Genome – wide association studies (GWAS) scan for hundreds or thousands of genetic differences in an attempt to link genome variations to particular traits and disease.
- Pharmacogenomics and rational drug design have led to customized medicines based primarily on a person's genotype.
- Gene therapy by transfer of cloned copies of functional alleles into target tissues is used to treat genetic disorders.

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THANK YOU