'' Role of gene therapy in hematological malignancy: review article.''

Manar Elhussiny, **Osama Elbaz Elagrody**[1], **Mohammed Mohammed Elsayed Elarman.**[2]

Prof. of Hematology, Mansoura Faculty of Medicine [1]

Ass. Prof. of Clinical Pathology, Mansoura Faculty of Medicine [2]

Corresponding author: Manar Elhussiny Mohammed Elmorsy Omar

Resident of Clinical Pathology, Mansoura University Hospitals - Faculty of Medicine

Email: Manarmm_2008@yahoo.com

ABSTRACT

Since cancer is the result of genetic mutations, it should be well suited for correction through gene therapy. Hematological malignancies in which human gene transfer has been performed are leukemias, lymphomas, graft-versus host disease after allogeneic bone marrow transplantation in leukemia, and multiple myeloma. Gene therapy may be used to induce or enhance an antitumor immunological reaction, to correct a genetic defect in the tumor cells, to render the malignant disease more susceptible to conventional therapies, to make the normal host cells more resistant to conventional therapies, or to track cells used for therapy. Gene therapy will probably be most valuable for the eradication of minimal residual disease after the use of conventional therapies. Gene therapy may improve the body's ability to fight cancer or make the cancer more sensitive to chemotherapy. It may be used to induce or enhance an antitumor immunological reaction, to correct a genetic defect in the tumor cells, to render the malignant disease more susceptible to conventional therapies, to make the normal host cells more resistant to conventional therapies, or to track cells used for therapy. Hematological malignancies in which human gene transfer has been performed are leukemias, lymphomas, risk after allogeneic bone marrow transplantation (BMT) in leukemia and multiple myeloma.

Keywords: Gene Therapy, Hematological Malignancy

INTRODUCTION

It is generally believed that tumors arise from single cells that due to accumulation of genetic lesions start to divide in abnormal manner. The process of tumor evolution usually starts with moderately increased proliferation but the end result is highly malignant cell population with the capacity for continuous cell division and with the ability to escape apoptosis and differentiation signals ***(Hanahan et al., 2000)***. The molecular characterization of congenital and acquired human disease over the past several decades has stimulated scientists and clinicians to use genetic therapy as a new and exciting possibility ***(Blau and Springer, 1995)***. By the 1990s, gene transfer technologies had progressed sufficiently to offer real hope for successful widespread clinical application ***(Kerr and Mule, 1994).*** The causal relationship between the expression of a mutated and dysregulated oncogene and certain malignancies has stimulated the search for a way to specifically turn off the expression of these genes ***(Zhang, 1996).***

Gene Therapy

Treatment of a disease by expression of one or multiple specific genes in a cell or group of cells. The production of the desired gene product corrects the genetic defect or alters cellular function **(Salmon et al, 1993).**

There are two types:

• Somatic gene therapy: expression of genes in differentiated somatic cells.

• Germline therapy: expression of genes in fertilized human oocytes or embryonic stem cells **(Larin et al., 2002).**

The Evaluation of Gene Transfer Methods:

• **Efficacy**: transfection quality (transient or stable), transfection efficiency, tropism (potential of organ-specific specific gene transfers), biological efficacy (expression of gene products).

• **Safety:** tolerance, adverse effects, immunogenicity.

• **Production effort**, costs and compliance criteria *(Baum et al., 2003).*

Vectors for Gene Therapy

A: Viral Vectors

Al: Adenoviral Vectors; Adenoviruses (Ad) contain a linear double-stranded DNA packaged in an icosahedral, non-enveloped capsid with fiber-like projections from each of the 12 vertices **(Kojaoghlanian et al., 2003).**

A2: Adeno-associated Viral Vectors; Recombinant adeno-associated virus (rAAV) vectors are another major type of viral vectors currently used in gene therapy studies. AAV is plication-defective parvovirus that depends on a helper virus, either adenovirus or herpes virus, for its propagation during lytic infection **(Tal, 2000).**

A3: Retroviral Vectors; Retroviral vectors are based on retroviruses that comprise a large class of enveloped viruses that contain two identical single-stranded RNAs (7-11 kb) as the viral genome. The retroviridae family consists of seven genera: alpharetrovirus, betaretrovirus, gammaretrovirus, deltaretrovirus, epsilonretrovirus, lentivirus, and spumavims **(Pringle, 1999).**

B: Nonviral Vectors

Liposomes are the most studied types of non-viral vectors. Based on their charge, liposomes can be classified into two classes, positively charged or cationic liposomes and negatively charged or pH-sensitive liposomes **(Singhal and Huang, 1994).**

Cationic liposomes are commonly used for gene delivery and many types of formulations are available. They are made up of a cationic lipid and a neutral lipid **(Gao and Huang, 1995*)*.**

Obstacles and Solutions

Despite the development of vectors for gene delivery and a few successful gene therapy trials, problems in gene delivery and transgene expression remain to be solved before gene therapy can be used as a common practice.

A: Physical Barriers to Gene Delivery

Most conventional drugs can be delivered orally , intravenously or subcutaneously. However, genes delivered in viral vectors or complexed with liposomes have difficulty to reach target cells. because of viral vectors or DNA/liposome complexes are much larger in size than conventional drugs and therefore they cannot move freely inside a body. Regarding in vivo migration, rAAV is the only known type that can cross the blood vessel barrier because of the relatively small size **(Wang et al., 2005).**

B: Immunological Barriers

The immunological barriers to gene delivery can be divided into two categories, innate and adaptive immune responses. Although the adaptive immune response to viral vectors was recognized early on and investigated extensively, the innate immune response was greatly underestimated **(Yang et al., 1995).**

C: Transgene Expression Barriers

How to control therapeutic gene expression was ignored initially by the gene therapy community since some viral promoter showed high activity in cultured cells. In addition, the initial goal in gene therapy research was to demonstrate gene transfer and gene expression. It was later discovered that viral promoters can be attenuated by host cytokines and some retroviral promoters can be silenced following vector integration **(Barquinero et al., 2000) and (Sung and Bromberg, 2001).**

Hematological Malignancy

Leukemia and lymphomas are relatively common, affect all ages, and demonstrate extraordinary biologic, morphologic, and clinical heterogeneity. Advances in monoclonal antibody technology, cytogenetic, and molecular genetics have revealed that the hematopoietic-lymphoid system (HLS) is a heterogeneous population of cells organized into anatomically and functionally distinct compartments. These same techniques also have been applied to neoplasm and have provided insight into the diagnosis, classification, and pathogenesis of leukemia and lymphomas **(Lukes and Collins, 1992).**

LEUKEMIA

I) Myeloid leukemia

Acute myeloid leukemia

Acute myelogenous leukemia (AML) is a clonal, malignant disease of hematopoietic tissue that is characterized by accumulation of abnormal (leukemic) blast cells, principally in the marrow, and impaired production of normal blood cells. Thus, the leukemic cell infiltration in marrow is accompanied, nearly invariably, by anemia and thrombocytopenia. The absolute neutrophil count may be low or normal, depending on the total white cell count **(Jemal et al., 2002).**

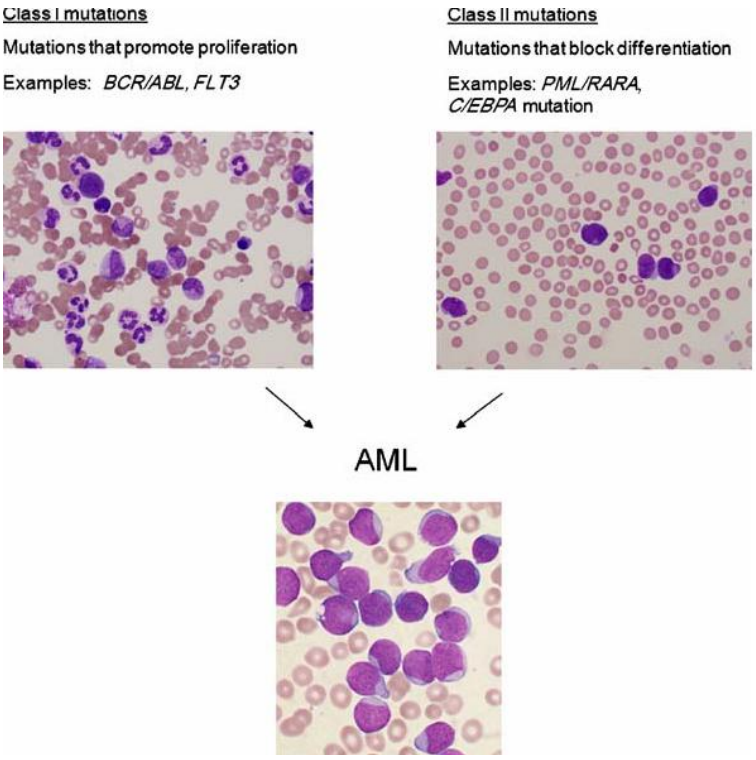

Figure (1): Several mutations are necessary for the development of acute myeloid leukemia (AML). Most AMLs have a mutation or translocation in a gene that promotes proliferation (class I mutation), as well as a mutation or translocation that alters a gene whose product promotes differentiation (class II mutation). The combination of blocked differentiation and abnormal proliferation results in AML. The resultant accumulation of blasts is shown in the photograph under "AML." The photograph under "class I mutations" is a peripheral blood smear from a patient with chronic myeloid leukemia; the photograph under "class II mutations" is a peripheral blood smear from a patient with acute promyelocytic leukemia. C/EBPα, CCAAT/enhancer binding protein-α; FLT3, fms-like tyrosine kinase-3; PML, promyelocytic leukemia; RARα, retinoic acid-α**.(Kelly, and Gilliland, 2002).**

Myelodysplastic syndromes (MDS)

Patients with 10% or more blasts in the bone marrow have a worse clinical outcome than do those with fewer blasts **(Greenberg et al., 1997).**

Because of the controversy as to whether chronic myelomonocytic leukemia (CMML) is a myelodysplastic or a myeloproliferative disease, this disorder has been placed in a newly created disease group, MDS/MPD (**Greenberg et al., 1997).**

Myelodysplastic / myeloproliferative diseases (MDS/MPD)

Table (1) WHO classification of the myelodysplastic/myeloproliferative diseases **(Bennett et al., 1994) and (James, 2002):**

* Chronic myelomonocytic leukemia
*Atypical chronic myeloid leukemia
* Juvenile myelomonocytic leukemia
*Myelodysplastic/myeloproliferative disease, unclassifiable

Chronic myelogenous leukemia (CML)

WHO reported that CML is a myeloproliferative disease that is characterized by the invariable presence of the Ph chromosome or the BCR/ABL fusion gene. Although in most cases the diagnosis is easily made from morphologic evaluation of the blood smear, confirmation by genetic studies is essential, particularly in view of the advent of therapy that targets the BCR/ABL fusion protein **(Druker et al., 2000).**

Mast cell disease (mastocytosis)

Mast cells originate from a bone marrow hematopoietic stem cell and demonstrate a number of characteristics that indicate they are myeloid cells. Mastocytosis includes a heterogeneous group of diseases characterized by abnormal growth and accumulation of mast cells in one or more organ systems **(Boissan et al., 2000).**

Recent data suggest that most variants of systemic mast cell disease are clonal, the protooncogene that encodes the tyrosine kinase receptor for stem cell factor, is usually present **(Longley et al., 1999).**

II) Lymphoblastic leukemia

Acute lymphoblastic leukemia

Clinical Features at Diagnosis

The most favorable age at presentation is 1–9 years, and white blood count is <50,000/mm3 **(Pui, and Evans, 1998).**

An elevated white count greater than 50,000/µl at presentation is often seen in children and adolescents with T-cell leukemia, those with aggressive leukemia chromosomal abnormalities such as t(4:11) and t(9:22), and infants (Pui et al., 2002).

The poorer prognosis seen in infant ALL results from high-risk leukemia characteristics including a high white count (>50,000/mm3) and central nervous system involvement in association with a mixed-lineage leukemia (MLL; 11q23) gene rearrangement of the leukemia cell **(Biondi et al., 2000).**

Cytogenetics and Chromosome Number

Hyperdiploid leukemia refers to tumor cells having a modal number of 51–68 chromosomes, occurs in 25% to 30% of the cases of childhood ALL, and confers a favorable prognosis **(Look, et al., 1985).**

Translocations

The transfer of a segment of one chromosome to another chromosome results in a translocation. Translocations within the leukemia cell have long been known to infer response to therapy and prognosis. The most common translocation is t (12:21), occurring in 20% to 25% of childhood ALL cases. This translocation can only be detected using molecular analysis and confers a favorable prognosis. Another common translocation is t (1:19), with a frequency of 3% to 6% among childhood ALL cases. Less common translocations are t (4:11) and t(9:22), which both infer a poorer prognosis **(Raimondi, 1993).**

Genomic Abnormalities

Conventional cytogenetic studies were initially carried out in CLL in the 1980s, and these proved difficult as CLL cells have a very low proliferative index, and even with mitogens, metaphases are difficult to obtain **(Stilgenbauer et al., 2002).**

When analysis is possible, clonal chromosomal abnormalities are detected in approximately 50% of cases and it is likely that the normal karyotyping in the remaining cases is due to analysis of contaminating normal T cells **(Döhner et al., 2000) and (Novak et al., 2002).**

Of the 50% of cases with clonal abnormalities, one-half have one clonal abnormality, and the remainder have two or three clones **(Juliusson and Merup, 1998).**

III) Non-Hodgkin Lymphomas

Immunophenotypic and Genotypic Analysis

Leukocyte common antigen (CD45) is a reliable marker for identifying most hematopoietic or lymphoid neoplasms but can be negative in acute leukemia, plasma cell neoplasms, and anaplastic large cell lymphoma (ALCL) **(Weiss et al., 1993).**

Several markers that work well in paraffin, such as L-26 (CD20), polyclonal CD3, and UCHL-1 (CD45RO), are adequate to categorize most NHL as to their B-cell or T-cell lineage **(Chadburn, and Knowles, 1994).**

Gene therapy of hematological malignancy

The molecular characterization of congenital and acquired human disease over the past several decades has stimulated scientists and clinicians to envision genetic therapy as a new and exciting possibility **(Blau, and Springer, 1995).**

By the 1990s, gene transfer technologies had progressed sufficiently to offer real hope for successful widespread clinical application **(Kerr, and Mule, 1994**).

(1) Immunomodulatory gene therapy

(a) Tumor vaccines

The genetically modulated cancer vaccine represents a classic ex vivo strategy involving the transfection of cytokines or other immunomodulatory factors into cultured, lethally irradiated tumor cells in an effort to stimulate an immune response when the engineered cells are reinjected into the recipient, usually into the skin. In human hematological malignancies one problem is that the primary malignant cells are highly resistant to transduction by most available vectors **(Anderson, 1998).**

(b) Dendritic cells

Dendritic cells (DC), the major class of antigen-presenting cells, are capable of providing all of the signals necessary to activate a de novo immune response. DC are found in the skin and mucous membranes (Langerhans' cells), lymphoid tissue, bone marrow, and circulating blood. Once these cells encounter a potential antigen, they interact with T and B cells to induce the immunological response cascade that is manifest as acquired cellular and humoral immunity **(Reinhard et al., 2001.)**

(c) Gene-modified cytotoxic T cells

Cytotoxic T lymphocytes (CTL) recognize processed intracellular proteins presented as short peptide fragments (together with MHC molecules) on the cell surface. Therefore, internal proteins unique to the malignant clone may act as tumor-specific antigens for CTL. Hematological malignancies may express a number of tumor-specific proteins, such as immunoglobulin or T cell receptor idiotypes, mutated oncoproteins, or fusion proteins generated by chromosomal translocation, or viral proteins **(Yee et al., 1997).**

(d) Cytokine gene therapy

While administration of recombinant cytokine(s) can produce to some extent antitumor effects or immunological changes in patients, the toxic reactions preclude the use of large amounts of the recombinant proteins. Therefore, researchers attempted to transfer cytokine gene(s) into tumors and to secrete the cytokine into the vicinity of tumors **(Wittig et al., 2001).**

(2) molecular chemotherapy

(a)Drug resistance gene therapy

It is now established that tumor cells can acquire drug resistance by alterations of pathways involved in the regulation of apoptosis and that failure to activate this pathway in cancer cells may confer resistance to chemotherapy. This resistance to drug-induced apoptosis is likely to play an important role in tumors that are refractory to chemotherapy **(Nemunaitis, and Cunningham, 2002).**

(b) Gene therapy combined with chemotherapy / chemoprotection

A gene therapy approach combines two strategies to confer chemoprotection to vulnerable tissues whilst sensitizing malignant tissues. O6-alkylguanine-DNA alkyl transferase (ATase) can confer protection against O6-alkylating agents. Mutant forms of ATase are resistant to the effects of soluble analogues of O6- alkylation such as O6-benzylguanine **(Hobin and Fairbairn, 2002).**

(3) Induction of tumor cell apoptosis

Components of the signaling network of apoptosis (programmed cell death), which include ligands such as CD95, TNF, and TNF-related apoptosis-inducing ligand, as well as downstream molecules, such as caspases, Bcl-2 family members, and inhibitor-of-apoptosis proteins, which trigger and regulate apoptosis, are important targets for the development of cancer gene therapy (**Los et al., 2003).**

(4) Inactivation of products of oncogenes.

(a) Oncolytic viruses

Arming viruses with therapeutic transgenes and combining the traditional modalities of chemotherapy and radiation therapy with oncolytic viral therapy in the hope of reducing the chance of developing resistant tumor cell clones. Another approach to augmenting the antineoplastic effect of these viruses involves modulating the immune response to minimize antiviral immunity, while at the same time maximizing antitumor immunity (**Donahue et al., 2002)**

(b) Antisense oligonucleotides

The use of antisense oligonucleotides aims to block target proteins. Antisense oligonucleotides are short DNA or RNA fragments consisting of a sequence complementary to a mRNA. When such an oligonucleotide is introduced into/synthesized in a cell it forms a double strand together with the mRNA, blocking the reading of the mRNA at the ribosomes **(Anthony et al., 2005).**

Gene therapy for leukemia

Genetically modified autologous CML cells may be used to enhance the T cell response. It is likely that T cells present in most CML patients are anergic to autologous

CML cells because of the lack of appropriate co-stimulatory molecules, such as the B7.1 molecules on leukemic cells. Therefore, T cell recognition of leukemic cells only leads to partial T cell activation, with subsequent development of anergy **(Lim Coleman, 1997).**

Gene therapy for multiple myeloma (MM)

Gene transfer is increasingly applied in MM. Most of the gene therapy strategies currently investigated in MM are based on immunotherapy, since myeloma cells express tumor-associated antigens (TAAs) and both allogeneic and autologous immune responses have been shown **(Van Baren et al., 1999).**

Gene therapy for lymphoma

DNA immunization was proposed as an attractive alternative to protein immunization against B cell lymphoma **(Syrengelas et al., 1996).**

Transfer of genes encoding IL-2, IL-12, IFN-α, IFN-γ, and GM-CSF into lymphoma cells has shown first experimental results in vivo **(Wierda, and Kipps, 2000).**

Telomerase regulation in hematological cancers

Telomeres are nucleoprotein structures composed of tandem arrays of telomeric repeats (5′-TTAGGG-3′). They preserve genome integrity and prevent chromosome ends from being recognized as double-strand breaks avoiding the activation of DNA damage pathways that result in senescence and cell death. Telomeres of human somatic cells shorten gradually during successive cycles of cell division. This shortening leads to chromosomal instability and senescence **(Blackburn, 1992).**

Epigenetic therapies in hematological malignancies

Epigenetic refers to changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence, hence the name epi- (Greek: over; above) -genetics. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism (**Adrian Bird, 2007).**

Epigenetic treatment in myelodysplasticsyndromes

Epigenetically active drugs currently within clinical trials include histone deacetylase inhibitors (HDACi) and DNA methyltransferase (DNMT) inhibitors. 5-azacytidine and 5-aza-2′-deoxycytidine, are epigenetically active drugs for MDS therapy **(Andrea and Michael, 2008)**.

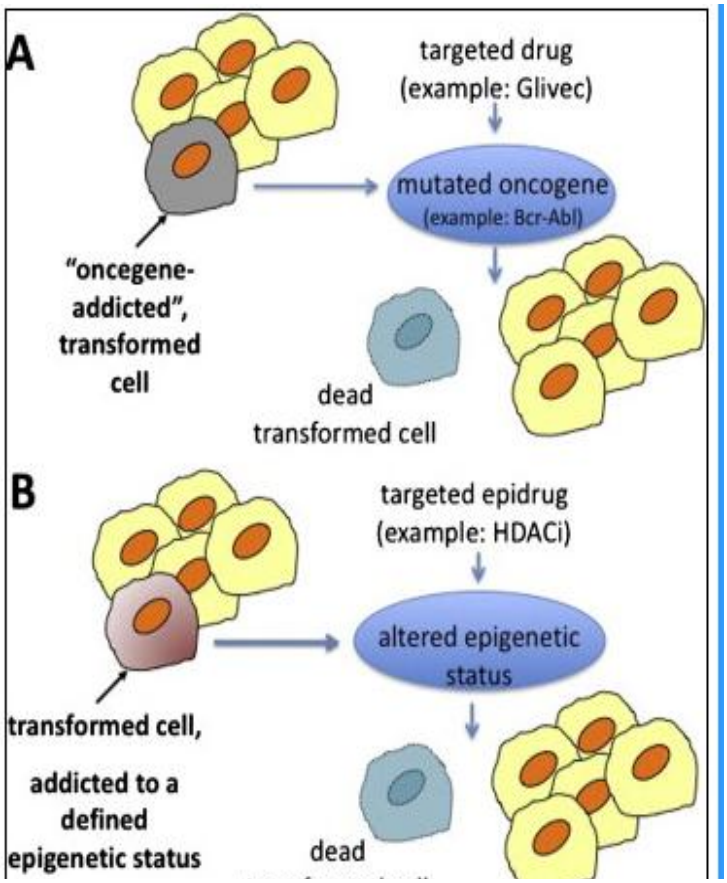

Figure 2: Targeted therapy as a paradigm for epigenetic therapy ('Epi-cures'). (A) General rationale for the use of molecularly targeted agents in cancer (example: Glivec in leukaemias). (B) Epigenetic therapies must identify tumour-specific epigenetic alterations, which lead to the use of the appropriate epigenetic drugs in a targeted way **(Altucci and Minucci ,2009).**

Molecularly targeted therapies in myelodysplastic syndromes and acute myeloid leukemias

Receptor tyrosine kinase inhibitors

FMS-like tyrosine kinase 3 (FLT3) receptor is a membrane-bound receptor tyrosine kinase which is crucial for the maintenance, proliferation, and differentiation of hematopoiesis . FLT3 is expressed by normal myeloid and patients **(Carow et al., 1996)**

FLT3 inhibitors

Several small-molecule tyrosine kinase inhibitors (TKIs) with varying specificity for FLT3 have been developed, including CEP-701, CEP-5214, SU5416, SU5614, SU11248, MLN518 (CT53518), N-benzoylstaurosporine (PKC412), L-000021649, and AG1295 . Some of these compounds have demonstrated promising clinical results in AML and myeloproliferative diseases (**Chalandon, and Schwaller, 2005*).***

Notch inhibitors in the therapy of hematological malignancies

Notch is a family of transmembrane proteins that function both as cell surface receptors and transcription regulators. The first gene of this family was cloned in the mid-1980s (**Wharton et al., 1985).**

The first human gene of this family, Notch-1 was discovered in 1991 through the analysis of the chromosomal translocation t(7;9)(q34;q34.3) observed in patients with T-cell acute lymphoblastic leukemia (T-ALL) (**Ellisen et al., 1991).**

T-cell acute lymphoblastic leukemia (T-ALL)

In human leukemias, Notch-1 activation was initially demonstrated in T-ALL harboring the t(7;9)(q34;q34.3), a rare chromosomal translocation identified in less than 1% of T-ALL cases. As a result of this rearrangement a truncated Notch-1 gene is juxtaposed next to the T-cell receptor β locus, leading to the ligand-independent aberrant expression of a constitutively active form of Notch-1 **(Ellisen et al., 1991).**

B-cell malignancies

The majority of B-cell malignancies were reported to overexpress Notch proteins. Thus, high level of active Notch was demonstrated in Hodgkin lymphoma, multiple myeloma (MM), B-cell chronic lymphocytic leukemia and weak but still detectable expression of Notch was observed in non-Hodgkin lymphoma cells (Nefedova et al., 2004).

References

- **Adrian B.** (2007): Perceptions of epigenetics. *Nature*, 447 (7143), 396–398.
- **Altucci L, and Minucci S. (2009):** Epigenetic therapies in haematological malignancies. *European Journal of Cancer*, 45(7), 1137-1145
- **Anderson WF.** (1998): Human gene therapy. Nature, 392, 25–30.
- **Andrea K. and Michael L**. (2008): Current status of epigenetic treatment in myelodysplastic syndromes. Ann Hematol, 87:601–611.
- **Barquinero J, Segovia JC, Ramirez M, Limon A, Guenechea G, Puig T, Briones J, Garcia J, and Bueren JA.** (2000): Efficient transduction of human

hematopoietic repopulating cells generating stable engraftment of transgene-expressing cells in NOD/SCID mice. *Blood, 9,* 3085-93.

- **Baum C, Dmann J, and Li Z.** (2003): Side effects of retroviral gene transfer into hematopoietic stem cells. *Blood,* 101, 2099–114.

- **Biondi I, Cimino G, Pieters R, and Pui CH.** (2000): Biological and therapeutic aspects of infant leukemia. *Blood*, 96: 24–33.

- **Blackburn EH.** (1992): Telomerases, *Annu. Rev. Bioche.,* 61, 113–129.

- **Blau HM, and Springer ML.** (1995): Gene therapy—a novel form of drug delivery. *N Engl J Med.,* 333, 1204–1207.

- **Boissan M, Feger F, Guillosson JJ, and Arock M.** (2000): c-Kit and c-kit mutations in mastocytosis and other hematologicaldiseases. *J Leukoc Biol.*, 67, 135-148.

- **Carow CE, Levenstein M, Kaufmann SH, Chen J, Amin S, Rockwell P, Witte L, Borowitz MJ, Civin CI ,and Small D.** (1996): Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. Blood, 87(3), 1089–1096.

- **Chadburn A, and Knowles DM. (1994):** Paraffin-resistant antigens detectable by antibodies L26 and polyclonal CD3 predict the B- or T-cell lineage of 95% of diffuse aggressive non-Hodgkin's lymphomas. *Am J Clin Pathol.*, 102, 284–291.

- **Chalandon Y and Schwaller J.** (2005): Targeting mutated protein tyrosine kinases and their signaling pathways in hematologic malignancies. Haematologica, 90(7), 949–968.

- **Döhner H, Stilgenbauer S, and Benner A.** (2000): Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med.*, 343, 1910–1916.

- **Donahue JM, Mullen JT,and Tanabe KK.** (2002): Viral oncolysis. Surg Oncol Clin North Am., 11, 661–680.

- **Druker BJ, and Lydon NB.** (2000): Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J Clin Invest.*, 105, 3-7.

- **Ellisen L, Bird J, West D, Soreng A, Reynolds T, Smith S, and Sklar J.** (1991): TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms, 66**,** 649–661.

- **Gao X, and Huang L.** (1995): Cationic liposome-mediated genetransfer. *Gene Ther.*, 710-22.

- **Greenberg P, Cox C, and Le Beau MM.** (1997): International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood,* 89, 2079-2088.

- **Hanahan D and Weinberg RA.** (2000): The hallmarks of cancer, 100, 57-70.
- **Hobin DA,and Fairbairn LJ.** (2002): Genetic chemoprotection with mutant O6-alkylguanine- DNA-alkyltransferases. Curr Gene Ther., 2, 1–8.
- **James W, Vardiman N, Lee H, and Richard D.** (2002): The orld Health Organization (WHO) classification of the myeloid_neoplasms. *Blood*, 100, 2292-2302.
- **Jemal A, Thomas A, Murray T, and Thun M.** (2002): Cancer statistics. *CA Cancer J Clin.*, 52 (1), 23–47.
- **Juliusson G, and Merup M.** (1998): Cytogenetics in chronic lymphocytic leukemia. *Semin Oncol.*, 25, 192–196.
- **Kelly LM, and Gilliland DG.** (2002): Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet*, 3, 179–198**.**
- **Kerr WG, and Mule JJ.** (1994): Gene therapy: current status and future prospects. *J Leukoc Biol.*, 56, 210–214.
- **Kojaoghlanian T, Flomenberg R, and Horwitz MS.** (2003): The Impact of Adenovirus Infection on the Immunocompromised Host. *Rev Med Virol.*, 13, 155 171.
- **Larin, Z, and Mejia JE.** (2002): Advances in human artificial chromosome technology. *Trends in hematology*, 18, 313-319.
- **Lim SH and Coleman S** (1997): Chronic myeloid leukemia as an immunological target. Am J Hematol., 54, 61–67.
- **Longley BJ, Metcalfe DD, and Tharp M.** (1999): Activating and dominant inactivating c-KIT catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci U S A*, 96, 1609-1614.
- **Look Roberson PK, Williams DL, Rivera G, Bowman WP, and Pui CH.** (1985): Prognostic importance of blast cell DNA content in childhood acute lymphoblastic leukemia. *Blood***,** 65, 1079–1086.
- **Los M, Burek CJ, Stroh C, Benedyk K, Hug H and Mackiewicz A.** (2003): Anticancer drugs of tomorrow: apoptotic pathways as targets for drug design. Drug Discov Today, 8, 67–77.
- **Lukes RJ, and Collins RD.** (1992): Tumors of the hematopoietic system. Atlas of tumor pathology, 2nd series, fascicle 28. Washington, DC: Armed Forces Institute of Pathology, 1–38.
- **Nefedova Y, Cheng P, Alsina M, Dalton W and Gabrilovich D.** (2004): Involvement of Notch-1 signaling in bone marrow stroma-mediated de novo drug

resistance of myeloma and other malignant lymphoid cell lines, *Blood*, 103, 3503–3510.

- **Nemunaitis J,and Cunningham C.** (2002): Emerging new therapies for chemotherapy-resistant cancer using adenoviral vectors. Drug Resist Updat, 5, 34–46.

- **Novak U, Oppliger Leibundgut E, and Hager J.** (2002): A high-resolution allelotype of B-cell chronic lymphocytic leukemia (B-CLL). *Blood*, 100, 1787–1794.

- **Pringle CR.** (1999): Virus taxonomy-1999. The universal system of virus taxonomy, updated to include the new proposals ratified by the International Committee on Taxonomy of Viruses during 1998. *Arch Virol.*, 144, 421 -9.

- **Pui C, and Evans WE.** (1998): Acute lymphoblastic leukemia. *New England Journal of Medicine*, 339, 605–615.

- **Pui C, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, and Kamps W.** (2002): Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. *Lancet*, 359, 1909–1915.

- **Raimondi M.** (1993): Current status of cytogenetic research in childhood acute lymphoblastic leukemia. *Blood*, 81, 2237–2251.

- **Reinhard G, Märten A, Kiske M, Feil F, Bieber T,and Schmidt-Wolf IGH.** (2001): Generation of dendritic-cell based vaccines for cancer gene therapy. Br J Cancer, 86, 1529–1533.

- **Salmons B, and Gunzburg WH.** (1993): "Targeting of retroviral vectors for gene therapy ". Human Gene Therapy, 4(2), 129-141.

- **Singhal A, and Huang L.** (1994): Gene transfer in mammalian cells uisng liposomes as carriers. In Gene Therapeutics, J. A. Wolff, ed. (Boston, Birkhauser), 118-142

- **Stilgenbauer S, Bullinger L, and Lichter P.** (2002): Genetics of chronic lymphocytic leukemia: genomic aberrations and VH gene mutation status in pathogenesis and clinical course. *Leukemia*, 16, 993–1007.

- **Sung RS, Qin L, and Bromberg JS.** (2001): TNF-alpha and IFN-gamma induced by innate anti-adenoviral immune responses inhibit adenovirus-mediated transgene expression. *Mol Ther.*, 5, *151-61.*

- **Syrengelas AD, Chen TT and Levy R.** (1996): DNA immunization induces protective immunity against B-cell lymphoma. Nat Med., 2, 1038–1041.

- **Tal J.** (2000): Adeno-associated virus-based vectors in gene therapy. *J Biomed Sci.*, 7, 279-91.

- **Van Baren N, Brasseur F, Godelaine D, Hames G, Ferrant A, Lehmann F, Andre M, Ravoet C, Doyen C, Spagnoli GC, Bakkus M, Thielemans K and Boon T.** (1999): Genes encoding tumor-specific antigens are expressed in human myeloma cells. Blood, 94, 1156–1164

- **Wang Z, Zhu T, Qiao C, Zhou L, Wang B, Zhang J, Chen C, Li J, and Xiao X.** (2005): Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. *Nat Biotechnol*, 23, 321-8

- **Weiss LM, Arber DA, and Chang KL.** (1993): CD45. A review. *Appl Immunohistochem Mol Morphol*, 1, 166–181.

- **Wharton K, Johansen K, Xu T, and Artavanis-Tsakonas S.** (1985): Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats, 43, 567–581

- **Wierda WG, and Kipps TJ.** (2000): Gene therapy of hematologic malignancies. Semin Oncol., 27, 502–511.

- **Wittig B, Märten A, Dorbic T, Weineck S, Min H, Niemitz S, Trojaneck B, Flieger D, Kruopis S, Albers A, Löffel J, Albers P, Müller S, Sauerbruch T, Bieber T, Huhn D,and Schmidt-Wolf IGH.** (2001): Therapeutic vaccination against metastatic carci- noma by expression-modulated and immuno-modified autol- ogous tumor cells: a first clinical phase/II trial. Hum Gene Ther., 12, 267–278.

- **Yang Y, Li Q, Ertl HC, and Wilson JM.** (1995): Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *J Virol*, *69*, 2004-15.

- **Yee C, Riddell SR and Greenberg PD.** (1997): Prospects for adoptive T cell therapy. Curr Opin Immunol., 9, 702–708.

- **Zhang WW. (1996)**: Antisense oncogene and tumor suppressor gene therapy of cancer. J Mol Med., 74, 191–204.