INTRODUCTION

Hematological malignancies are the types of cancer that affect blood, bone marrow and lymph nodes. As the three are intimately connected through the immune system, a disease affecting one of the three will often affect the others as well ***(Kovtum et al., 2010).***

Although lymphoma is technically a disease of the lymph nodes, it often spreads to the bone marrow, affecting the blood and occasionally producing a paraprotein, evidence was provided suggesting chemotherapy as standard treatment for leukemia could only eliminate the actively reproducing tumor cells, whilst the immune system was required to eliminated minimal residual disease. This historical overview allows an understanding of the reasons why IT for hematological conditions has been much centered on immunotherapy ***(Lenz et al., 2005).***

Bone marrow transplantation lessons learnt from this procedure have led to the birth of new strategies for the treatment of malignancies via cell driven therapies ***(Thomas et al., 2010).***

Radioimmunotherapy (RIT) has emerged as one of the most promising treatment options, particularly for hematologic

malignancies. However, this approach has generally been limited by a suboptimal therapeutic index (target-to-nontarget ratio) and an inability to deliver sufficient radiation doses to tumors selectively ***(Vallera et al., 2004).***

Pretargeted RIT (PRIT) circumvents these limitations by separating the targeting vehicle from the subsequently administered therapeutic radioisotope, which binds to the tumor-localized antibody or is quickly excreted if unbound ***(Golay et al., 2005).***

A growing number of preclinical proof-of-principle studies demonstrate that PRIT is feasible and safe and provides improved directed radionuclide delivery to malignant cells compared with conventional RIT while sparing normal cells from nonspecific radiotoxicity ***(Lopus et al., 2010).***

Early phase clinical studies corroborate these preclinical findings and suggest better efficacy and lesser toxicities in patients with hematologic and other malignancies, with continued research PRIT -based treatment strategies promise to become cornerstones to improve outcomes for cancer patients despite their complexities ***(Loo et al., 2005).***

Immunotoxins and antibody-drug conjugates are protein-based drugs combining a target-specific binding domain with a cytotoxic domain. Such compounds are

potentially therapeutic against diseases including cancer, and several clinical trials have shown encouraging results. Although the targeted elimination of malignant cells is elegant concept, there are numerous practical challenges that limit conjugates' therapeutic use, including inefficient cellular uptake, low cytotoxicity and off-target effects ***(Ikeda et al., 2009).***

The approval of monoclonal antibodies (MAbs) as antibody-targeted therapy in the management of patients with hematologic malignancies has led to new treatment options for this group of patients. The ability to target antibodies to novel functional receptors can increase their therapeutic efficacy ***(Borello et al., 2002).***

Improvements in the identification of tumour-associated antigens and in our understanding anti-tumour immune responses have revived interest in the use of therapeutic cancer vaccination. Due to their unique characteristics, hematologic malignancies represent an ideal target for vaccine based therapeutic interventions ***(Mitchell et al., 2002).***

AIM OF THE WORK

To highlight the role of immunotherapy as a new promising strategy in treatment of hematological malignancies.

HEMATOLOGICAL MALIGNANCIES

The hematological malignancies are clonal diseases that derive from a single cell in the marrow or peripheral lymphoid tissue which has undergone a genetic alteration**.** Leukemias and lymphomas are relatively common, affect all ages and demonstrate extra-ordinary biologic, morphologic and clinical heterogenecity ***(Bordoni et al., 2007).***

Leukemia

The leukemias are a group of disorders characterized by the accumulation of malignant white cells in bone marrow (BM) and peripheral blood (PB). These abnormal cells cause symptoms because of bone marrow failure (i.e. anemia, thrombocytopenia, neutropenia) and infiltration of organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin or testes),the main classification is into four types: acute and chronic leukemias, which are further subdivided into lymphoid or myeloid ***(Hoffbrand et al., 2006).***

Acute leukemia

Acute leukemia is defined as the presence of over 20% of blasts cells in the bone marrow at the time of presentation. It can be diagnosed with even less than 20%

blasts if specific leukemia-associated cytogenetic or molecular genetic abnormalities are present. If untreated these diseases are rapidly fatal but they are also easier to cure than chronic leukemias ***(Bain, 2003)***.

Acute leukemias are usually aggressive diseases in which malignant transformation occurs in the hemopoietic stem cell or early progenitors. The genetic damage is believed to involve several biochemical steps resulting in increased rate of proliferation , reduced apoptosis ,block in cellular differentiation.It is further subdivided into acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) and acute biphenotypic leukemia on the basis of whether the blasts are shown to be myeloblasts or lymphoblasts ***(Hoffbrand et al., 2006).***

1] Acute myeloid leukemia

AML is a clonal, malignant disease of hematopoietic tissue that is characterized by accumulation of abnormal blast cells (principally in marrow), impaired production of normal blood cells, thus, the leukemic cell infiltration in marrow is accompanied by anemia and thrombocytopenia. AML is the common form of acute leukemia in adults and is increasingly common with age. But it forms a minor fraction (10-15%) of the leukemias in children**.** An important

distinction is between primary AML which appears to raise de novo and secondary AML which can develop from myelodysplasia (MDS) and other hematological diseases such as the myeloproliferative diseases (MPD) or follow previous treatment with chemotherapy ***(Hoffbrand et al., 2006).***

Different mechanisms related to development of AML as shown in table (1):

1) Inappropriate Proliferation: The Role of Signaling Molecules

Activation of receptor and intracellular protein tyrosine kinases stimulates a cascade of phosphorylation-driven protein docking and recruitment events that leads to the alteration of transcription in the cell nucleus and the stimulation of cell cycle progression. Although cell proliferation is regulated by the presence of growth factors and adhesion signals in normal cells, it can be triggered in leukemic cells in a cell autonomous manner. This abnormal proliferation is often the result of mutations affecting proliferative signaling pathways ***(Steensma et al.,2005)***.

Activated kinases have become causally implicated in the pathogenesis of AML. The FLt3 tyrosine kinase is expressed in 30-75% of AML patients. The c-KIT tyrosine

kinase is expressed in 60%-80% of AML patients, The JAK2 kinase activated by the V617F point mutation present in hematological cases that can evolve into AML such as polycythemia vera, essential thrombocythemia and myelofibrosis. Activated JAK2 induces proliferation in part through engagement of the STAT family of transcriptional activators. The V617F JAK2 mutation is found in 5% of patients with MDS, suggesting that the mutation will also be found in patients who transform to frank AML ***(Steensma et al., 2005).***

2) Differentiation Blockade: The Role of Transcription Factors in AML

Transcription factors are commonly disrupted in AML either by their fusion as a result of chromosomal translocation or by point mutation. Factors affected by chromosomal rearrangement include the core binding factor complex, the retinoic acid receptor (RAR), the MLL protein, and Hox proteins. Point mutations in myeloid transcription factors, including C/EBPα and PU.1, may also lead to loss of normal myeloid differentiation in AML. Chimeric transcription factors often work as dominant negative forms of the original factor. Core binding factor (CBF) and retinoic acid receptor (RAR) fusions are prime examples of this.

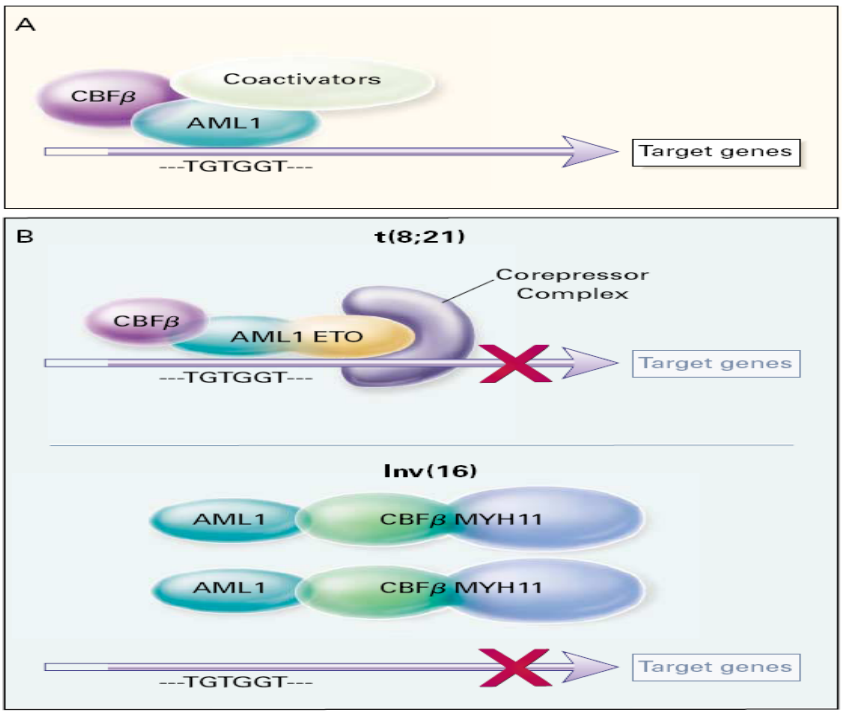

Figure (1): The AML1-CBF*β* Transcription Factor. In Panel A, in normal cells, heterodimeric AML1-CBF*β* transcription-factor complex binds to the DNA sequence TGTGGT in the transcriptional regulatory region of AML1-regulated target genes and activates transcription through the recruitment of co-activators. In Panel B, in AML cells with the t(8; 21) translocation, the N-terminal part of AML1 fuses with the C-terminal portion of ETO. The resultant chimeric protein continues to interact with CBF *β* and to bind to the core enhancer sequence; however, ETO recruits a nuclear co-repressor complex and results in the dominant repression of AML1-regulated target genes. Similarly, the CBF*β* -MYH11 chimeric protein encoded by the inv (16) mutation continues to interact with AML1; however, instead of allowing AML1 to interact with DNA, this chimeric protein recruits AML1 into functionally inactive complexes in the cytoplasm ***(Licht and Sternberg, 2005).***

Acute promyelocytic leukemia (APL)

APL is a clear-cut example of differentiation blockade in AML, is always associated with rearrangement of the retinoic acid receptor alpha (RARα). In over 98% of cases APL is associated with t (15; 17), which generates the PML-RARα fusion protein. The PLZF-RARα fusion associated with t (11; 17) represents less than 1% of cases and yields a retinoic acid-resistant form of disease. Rare fusions of RARα to STAT5b, nucleophosmin (a gene mutated in a substantial number of AML cases have been reported as well ***(Licht and Sternberg, 2005).***

3) Escape from Programmed Cell Death

The ability to evade apoptosis is critical to the development of a malignancy. Protein tyrosine kinase activation can have the dual effect of promoting cell proliferation and in addition enhancing cell survival by activating phosphatidylinositol 3-kinase (PI 3-kinase) signaling ***(Licht and Sternberg, 2005)***.

The phospholipid products of PI3-kinase activate the AKT serine/threonine kinase, and this kinase in turn phosphorylates BAD and releases the BCL-2 pro-survival molecule. In many instances the clinical outcome of patients with AML is correlated with altered levels of pro-

apoptotic and prosurvival molecules in leukemic cells ***(Licht and Sternberg, 2005).***

4) Self-Renewal

Unlike normal progenitor cells that are committed to a particular hematopoietic lineage, leukemic cells from patients with AML can undergo self-renewal rather than lineage specific commitment ***(Hope et al., 2004)***.

5) Loss of Cell Cycle Control

Deregulation of cell cycle control in AML may occur through multiple mechanisms. First, constitutive RAS/MAP kinase signaling leads to the activation of nuclear transcription factors that induce expression of cyclins. Activation of the AKT pathways can lead to degradation of the p27 CDK inhibitor. Mutation of p53, disruption of p53 function through suppression of ARF, disruption of the PML nuclear body function, or disruption of NPM ability to sequester MDM2 can all lead to decreased p53 levels and activity and the loss of G1 checkpoint control ***(Licht and Sternberg, 2005).***

6) Leukemia Cell Dissemination

The ability of the leukemic cell to egress from the marrow and invade tissues is poorly understood. t(8;21)-associated AML is associated with chloromas. High level

of selectin expression on the surface of leukemic cells is a negative prognostic marker in AML. Secretion of TNF and other cytokines by the leukemic blast can lead to increased expression of selectins, cadherins and other adhesion proteins on vascular endothelium, resulting in increased leukemia cell adhesion. Antibodies directed against these surface proteins block adhesion ***(Stucki et al., 2001).***

Table (1): Molecular lesions in AML associated malignant characteristics ***(Licht and Sternberg, 2005).***

Property	Autonomous Cell Proliferation	Differentiation Block	Escape from Apoptosis	Increased Self-Renewal	Loss of Cell Cycle Control	Dissemination
Molecular Lesion	Activating mutations: Flt3, Ras, c-Kit, c-FMS, Jak2, PTPN11 Inactivating mutation- NF1 Autocine loops: (Trk-A upregulation by RUNX1-MTG8)	Fusion transcription factors Retinoic acid receptor PML-RARα, PLZF-RARα Core binding factor RUNX1-MTG8 CBFβ-MYH11 RUNX1-EVI1 MLL-fusions Hox gene fusions and overexpression Point mutation of transcription factors Pu.1, C/EBPα, RUNX1 RTK inhibition of critical ractor expression 9 (Flt3 inhibits C/EBPα expression)	AKT pathway activation following RTK activation leads to Bad deactivation P53 mutations in AML of the elderly P53 dysregulation by fusion proteins, NPM mutation Bcl2 overexrepssion Survivin (IAP) overexpression	β catenin mutations Activation of Wnt Catenin pathway by fusion transcription factors Activated RTK pathways cooperate to induce self-renewal	P53 dysfunction Loss of Rb P15, P16 cyclin-dependent kinase gene methylation	TNF secretion by leukemic blasts stimulates endothelium. Increased selectin, cadherin and integrin expression encourage adhesion and egress through vessels

Classification of AML:

Classification is usually based on the morphological criteria of the FAB scheme which divides AML into eight variants (Table 5). These FAB subtypes are associated with characteristic patterns of cytochemical stains, immuno-phenotype and chromosomal changes, modified by the WHO classification in which at least 20% blasts cells are required ***(Hoffbrand et al., 2006).*** Specialized tests are needed to confirm the diagnosis of AML and to divide it into its different types. These tests include immunophenotyping (Table 2 and Table 4) and cytochemistry staining (Table 3).

Table (2): Immunologic phenotypes of AML ***(Hoffbrand et al., 2006).***

Type	Usually positive
Myeloblastic	CD11,CD13,CD15,CD33,CD117,HLA-DR
Myelomonocytic	CD11,CD13,CD14,CD15,CD32,CD33,HLA-DR
Erythroblastic	Glycophorin, spectrin, ABH antigens, carbonic anhydrase
Promyelocytic	CD11,CD13,CD15,CD33
Monocytic	CD11,CD13,CD14,CD33,HLA-DR
Megakaryoblastic	CD34,CD41,CD42,CD61,von Willebrand factor

Table (3): Specialized tests for ALL and AML ***(Hoffbrand et al., 2006).***

	All	**AML**
Cytochemistry		
Myeloperoxidase	-	+ (including Auer rods)
Sudan black	-	+ (including Auer rods)
Non- specific esterase	-	+ in M4,M5
Periodic acid - Schiff	+ (coarse block positivity)	+ (fine blocks in M6)
Acid phosphatase	+ in T-ALL (Golgi staining)	+ in M6 (diffuse)
Immunoglobulin and TCR Genes	• Precursor B-ALL: clonal rearrangement of Ig genes • T-ALL: clonal rearrangement of TCR genes	Germline configuration of immunoglobulin and TCR genes
Immunological markers (flow cytometry)		