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Aberrant Proliferative and Apoptotic Pathways in Acute Lymphoblastic Leukemia (ALL): Molecular Therapies to Overcome Chemo-Resistance

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1. Introduction

Adult acute lymphoblastic leukemia (ALL) is characterized by a high relapse rate, with the majority of patients developing chemo-resistance and ultimately dying of the disease with a 5-year survival rate of 40% (Faderl et al., 2010). Significant advances, however, have been made in cases carrying the acquired genetic alteration BCR-ABL (ALL-Ph+) targeted by tyrosine-kinase inhibitors (Ottmann & Pfeifer, 2009). Therefore, several studies have recently been carried out to look for additional, therapeutically exploitable, genetic lesions. Aberrant activation of signal transduction pathways (STP) implicated in proliferation and survival mechanisms are generally involved in leukemogenesis and drug resistance (Zhao et al., 2010). Genes in the PI3K/PTEN/AKT/mTOR, RAS/RAF/MEK/ERK, and Jak/STAT pathways are frequently mutated and their expression is often altered in hematopoietic malignancies, including ALL (McCubrey et al., 2011; Steelman et al. 2008). In addition, deregulation of survival mechanisms may confer chemo-resistance to leukemic cells, particularly involving alterations of the Bcl-2 signaling cascade, which may represent one of the most important, potentially druggable, pathways for therapeutic intervention in ALL.
Starting from our studies on chemo-resistance in ALL, particularly on multidrug resistance (MDR1) expression and prognostic significance, in this chapter we will illustrate the major pathways aberrantly activated in ALL - PI3K/PTEN/AKT/mTOR, RAF/RAS/MEK/ERK, and the Bcl-2 family of proteins - with the ultimate goal of summarizing novel targets for

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molecular therapies, especially in resistant/refractory cases. Controversies on and potential benefits of novel approaches based on STP inhibitors will be discussed. Epigenetic changes will be examined to address their importance in association with post-translational modifications. Future perspectives will encompass review of Reverse Phase Protein Arrays (RPPA) as a promising new tool for translational studies in clinical sample series, helping define the functional proteomic profile at steady-state; this approach may lead to the identification of aberrant molecular target(s), possibly guiding the choice of specific molecular therapies, on one hand, and leading to the elucidation of post-treatment changes, on the other, with the overall objective of improving the strategies currently employed to counteract leukemia chemo- and targeted drug-resistance.

2. Multidrug resistance

Chemo-resistance has been one of the major problems in cancer treatment, contributing to therapeutic failure, particularly in leukemia patients treated with regimens containing drugs such as anthracyclines, vinca alkaloids, or epipodophyllins, all modulated in their intracellular retention by the P-glycoprotein 170 (P-gp) product of the mdr-1 gene (Longley et al., 2005). An expanding number of proteins with a prominent role in MDR-dependent mechanisms of accelerated drug efflux and potentially implicated in leukemia drug resistance have been described: the multidrug resistance-associated protein 1 (MRP1), the lung-resistance protein (LRP) and the transporter breast cancer resistance protein (BCRP) (Chen, 2010). MRP1 is a protein distinct from MDR1, with different substrate specificity (amphophilic anions or glutathione-conjugated) and different susceptibility to drugs that can restore chemotherapy sensitivity (butathionine, which depletes intracellular glutathione content). LRP is a component of the major vault protein complex that appears to be involved in nuclear-cytoplasmic trafficking and in promoting drug redistribution away from the nucleus. BCRP is another member of the ATP-binding cassette protein involved in the MDR phenotype. In our previous studies (Tafuri et al., 2002) we have established the frequency and biologic–clinical significance of MDR1 expression and function and of MDR-associated proteins in *de novo* adult ALL. Our analysis was performed in the context of the ALL0496 study of the GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto) cooperative group. This prospective multicenter study enrolled a large series of uniformly treated *de novo* adult ALL patients, providing a unique opportunity to investigate the prognostic influence of MDR1 on CR achievement and clinical outcome, in context with other clinical and biological factors. Our results indicate that MDR1 is expressed in approximately 20% of patients enrolled in the study, playing a statistically significant unfavorable prognostic role on CR achievement at both univariate and multivariate analysis; in addition, together with age and CD34 expression, MDR1 expression significantly predicted response failure. Since MDR1 expression was described as an independent predictor of CR in patients with *de novo* adult ALL, additional studies (Gregorj et al., 2005) were performed by our group to investigate the prognostic role of other proteins implicated in drug efflux mechanisms in ALL. Analysis of primary ALL samples evaluated for the expression of MRP1 and BCRP found these proteins expressed in 51.3% and 72.9% of the cases, respectively. BCRP expression was associated with age (P=0.027), but not with MDR1 expression. Neither BCRP nor MRP1 individually influenced CR achievement. In contrast, when their simultaneous co-expression was analyzed, the majority of double negative patients achieved CR (P=0.034). Alterations in drug-efflux due to aberrant ABC transporter protein expression in cancer cells

led to the development of P-gp inhibitors, tested in clinical trials with discouraging results. In fact, the combined use of the MDR-reversal agent cyclosporine was associated with unacceptable toxicity, while a second generation-drug, vaslapodar (PSC-833), had unpredictable pharmacokinetic interactions. Third-generation inhibitors (tariquidar XR9576, zosuquidar LY335979, laniquidar R101933, and ONT-093) have high potency and specificity for P-gp but no appreciable impact on cytochrome P450 3A4 drug metabolism and no clinically significant drug interactions with common chemotherapy agents. Overall, the proposed chemo-sensitizing activity of MDR-reversal agents evaluated in clinical trials failed to improve either response rate or survival in leukemia patients (Molnár et al., 2010).
Since advances in microarray and proteomic technologies, in conjunction with the development of novel small molecules therapeutically targeting molecular determinants of resistance in cancer cells, have opened new opportunities to combat chemo-resistance, studies have explored and characterized the signaling pathways involved in the regulation of tumor cell response to chemotherapy, mostly those involved in aberrant proliferation and prolonged survival.

3. Signal transduction pathways

Leukemic cells are frequently characterized by the constitutive activation of multiple signaling cascades, which ensures a proliferative and survival advantage over normal hematopoietic cells. In fact, several studies have shown that the PI3K/PTEN/AKT/mTOR, RAS/RAF/MEK/ERK, Notch1, and JAK/STAT pathways are frequently up-regulated in ALL (Brown et al., 2008). Herein, some of these key signaling are reviewed as potential molecular targets for specific therapeutic interventions in ALL (Fig.1).

3.1 PI3K/PTEN/AKT/mTOR pathway

The phosphoinositide 3-kinase (PI3K)/PTEN/AKT/mTOR pathway is a crucial signaling cascade involved in the control of cell growth, survival, proliferation, motility, apoptosis and autophagy (Fig. 1). This pathway plays a pivotal role in maintaining numerous physiological processes, such as nutrient uptake, ribosomal biogenesis and cellular metabolism (Brown et al., 2008). PI3K is activated by a variety of extracellular stimuli, including receptor tyrosine kinases (i.e. FLT3, EGFR, HER-2/neu) (Fathi et al., 2010). The activation of PI3K catalyzes the phosphatidylinositol-4,5-bisphosphate (PIP2) conversion to the phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the inner surface of the cell membrane, allowing for the recruitment of PDK1 and AKT to the membrane. AKT is an important mediator of the intracellular cascade, acting on multiple downstream targets to regulate proliferation and apoptotic signals. Downstream of AKT, a key protein is represented by mTOR, which regulates translation in response to nutrient levels (Fig.1). The major negative regulator of the PI3K/AKT/mTOR pathway is the lipid phosphatase PTEN (Phosphatase and tensin homologue deleted on chromosome 10).
The PI3K/PTEN/AKT/mTOR pathway is deregulated in several human cancers, including ALL. First, Barata et al. (2004) demonstrated that the PI3K pathway is essential for IL-7–mediated survival, activation, proliferation, and growth of T-ALL cells indicating the existence of a functional IL-7–mediated PI3K signaling cascade in T-ALL cells. Additional reports showed that the constitutive activation of PI3K/PTEN/AKT/mTOR pathway occurs frequently in childhood ALL and is crucial for blast survival (Avellino et al., 2005; Jotta et al., 2010). Aberrant activation of this signaling cascade can be induced by overproduction of

growth factors or chemokines, by loss of PTEN expression, or by mutations in PTEN or in other mediators downstream of growth factor receptors (RAS and PI3K itself). PTEN mutations, deletions or inactivation are found in many ALL cell lines and are particularly frequent in human T-ALL cell lines derived from relapsed patients (Jotta et al., 2010). Conversely, PTEN gene mutations have been reported in a small but significant subset of *de novo* T-ALL patients (Silva et al., 2008). The NOTCH1 receptor, which is activated by mutations in about 50% of T-ALL, inhibits PTEN expression that, in turn, leads to AKT activation and resistance to glucocorticoids. Additionally, the PI3K/PTEN/AKT/mTOR pathway has been proposed to be involved in the regulation of MDR-1 expression and drug resistance in ALL, as suggested by cleavage of the 170-kDa P-gp during PI3K inhibitor-induced apoptosis of VBL100 human T-lymphoblastoid CEM cells (Mantovani et al., 2006). All these findings lend significant support to the development of therapies targeting the PI3K/PTEN/AKT/mTOR pathway in ALL, particularly in T-ALL.

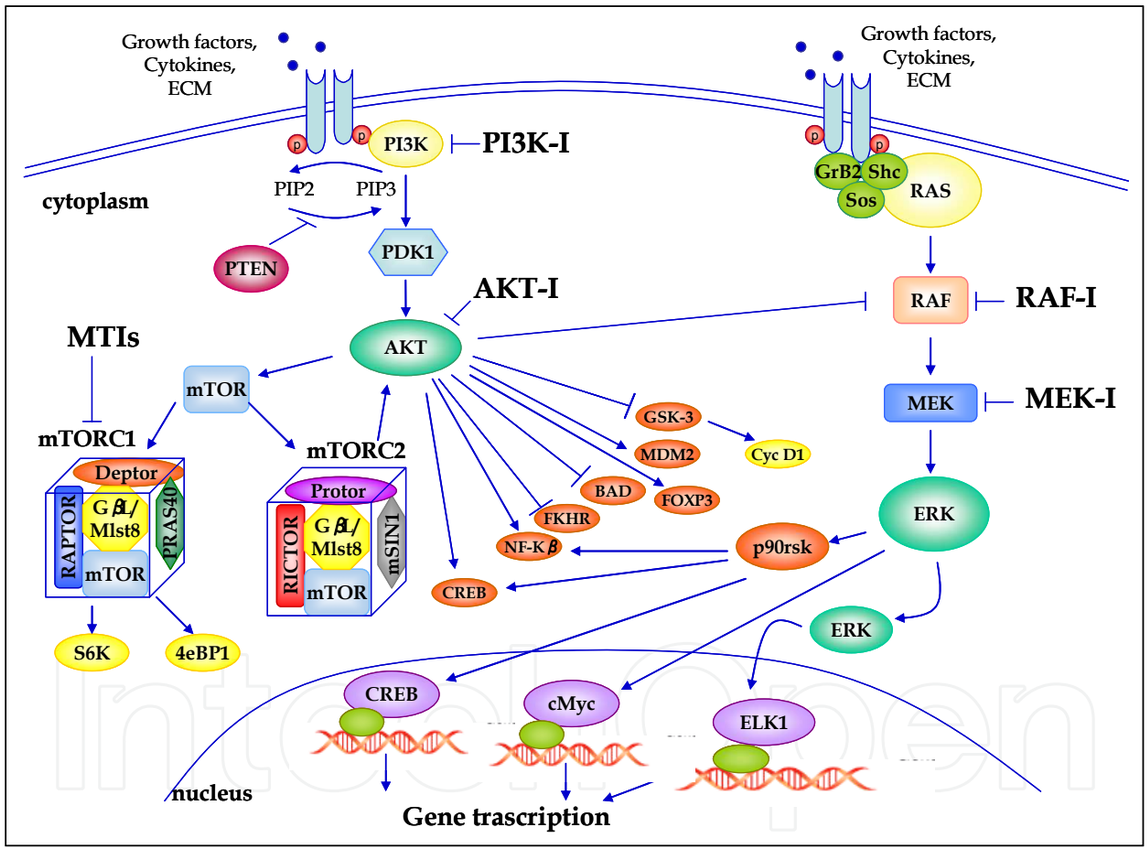

Fig. 1. PI3K/PTEN/AKT/mTOR and RAS/RAF/MEK/ERK pathways and their sites of interaction.

Inhibition of the PI3K signaling cascade can be achieved by targeting the pathway at different levels or simultaneously at multiple sites. In addition, however, these effects could be enhanced by also inhibiting crosstalking pathways. Specific inhibitors of PI3K, AKT and mTOR have shown significant promise in preclinical studies and are now under evaluation in clinical trials (Tab. 1) (Fathi et al., 2010; Levitzki & Klein, 2010; Zhao, 2010). Levy et al. (2009) reported that GSK690693, a pan-AKT kinase inhibitor, induces growth inhibition and

Inhibitor	Targets	Cancer Examined	Clinical Trials	Company
AR-12	PDK-1, PI3K, Akt	breast, colon, lung, prostate, lymphoma	Phase I	Arno Therapeutics
LY294002	PI3K, other related kinases	advanced hematological and advanced solid cancers	Preclinical	Lilly
CAL-101	PI3K (p110δ)	leukemias, lymphomas, myeloma	Phase I	Calistoga Pharmaceuticals
GDC-0941	PI3K (p110α), Flt3	lymphoma, NSCLC, breast, solid tumors	Phase I	PIramed Pharma/Roche/ Genetech
Wortmannin	PI3K, mTOR, DNA-PK, MAPK	advanced hematological and advanced solid cancers	Preclinical	
Perifosine (KRX- 0401)	Akt, MEK 1/2, ERK 1/2, JNK	multiple myeloma, leukemias, NSCLC, advance solid tumors	Phase I, II	Æterna Zentaris Inc./Keryx Biopharmaceuticals
triciribine (API-2)	Akt 1, 2, 3	AML, advanced hematological cancer	Phase I	VioQuest Pharmaceuticals
GSK690693	Akt1, 2, 3	leukemia, lymphoma	Phase I	GlaxoSmithKline
KP372-1	Akt, PDK-1, Flt3	leukemia, thyroid, H&N, glioma	Preclinical	QLT Inc.
VQD-002 (API-2)	Akt	NSCLC, leukemias, lymphomas, prostate	Phase I, II	VioQuest Pharmaceuticals
A-443654	Akt	hematological and solid cancers	Preclinical	Abbott Laboratories
Rapamycin (Sirolimus)	mTORC1	advanced hematological, advanced solid tumors, HIV, AIDS related malignancies	Phase I, II	Wyeth/Pfizer
CCI-779 (Torisel®, Temsirolimus)	mTORC1	leukemia, lymphoma, NSCLC, prostate, colorectal, renal	Phase I, II	Wyeth/Pfizer
RAD001 (Afinitor®, Everolimus)	mTORC1 mTORC2	cervical, renal, HCC, leukemia, lymphoma	Phase I, II	Novartis
AP-23573 (Ridaforolimus, Deforolimus)	mTORC1	advanced hematological cancer, prostate, endometrial	Phase I, II	Ariad/Merck
AZD-8055	mTORC1 mTORC2	advanced solid tumors, lymphomas, HCC	Phase I, II	AstraZeneca
OSI-027	mTORC1 mTORC2	advanced solid tumors, lymphomas	Phase I	OSI Pharmaceuticals
INK-128	mTORC1 mTORC2	advanced cancers, multiple myeloma, Waldenstrom macroglobulinemia	Phase I	Intellikine
PP-242	mTORC1 mTORC2		Phase I	UCSF

Table 1. PI3K/PTEN/AKT/mTOR inhibitors in hematological malignancies.

apoptosis of most of the ALL cell lines tested (89%), and exerts a selective antiproliferative effect on malignant cells (lack of effects on normal human CD4+ peripheral T lymphocytes). Much attention has been recently focused on blocking mTOR. This component of PI3K signaling cascade can form two distinct complexes termed mTORC1 and mTORC2 (Bjornsti & Houghton, 2004; Ciuffreda et al., 2010; Steelman et al., 2008). The first complex, composed

of mTOR, GβL/Mlst8, PRAS40, RAPTOR and Deptor, is sensitive to the mTOR inhibitors (MTIs), such as rapamycin and its analogs RAD001, CCI-779 and AP23573 (rapalogs). Conversely, mTORC2 complex is generated when mTOR is associated with GβL, mSIN1, RICTOR, and PROTOR/PRR5. Rapamycin and its analogs are in this case unable to disrupt mTORC2 activity. However, it has been demonstrated that prolonged exposure and/or higher concentrations of MTIs may inhibit mTORC2. MTIs are currently in various stages of development for treatment of human cancer, as they showed great preclinical promise in the treatment of many cancer types, including ALL (Chappell et al., 2011). A recent ongoing phase I/II study from Amadori et al. performed in acute myeloid leukemia (AML) by combining the RAD001 with clofarabine, has been preliminary reported with encouraging results in a poor prognosis patient population overexpressing the drug-target (Amadori et al., 2010). It has been reported that inhibition of mTOR by rapamicin leads to apoptosis of blasts from ALL patients of both B-cell and T-cell origin (Avellino et al., 2005). The efficacy of CCI-779 (temsirolimus) in primary human ALL was also evaluated by Teachey et al. (2006), who demonstrated its activity using both NOD/SCID xenograft models and bone marrow-derived stromal cell co-culture systems. They observed that CCI-779 induced a dramatic decrease in cell proliferation and an increase of apoptosis in lymphoblasts from adult B-ALL patients cultured on bone marrow stroma. Moreover, in a NOD/SCID xenograft model they found that CCI-779 showed a decrease in peripheral blood blast counts and in splenomegaly. More recently, Batista et al. (2011) reported that inhibition of mTOR by CCI-779 markedly potentiated the anti-leukemic effects of dexamethasone and doxorubicin in primary T-ALL cells, showing in addition highly synergistic interactions in combination with other specific inhibitors of the PI3K cascade or with inhibitors of JAK3 signaling. According to the observation that ALL cells are frequently characterized by the deregulation of the apoptotic machinery, we explored *in vitro* a novel therapeutic strategy for ALL cells based on the combined inhibition of mTOR signaling and Bcl-2 activity, using CCI-779 and ABT-737, a small molecule BH3-mimetic that inhibits Bcl-2/Bcl-xL proteins (Iacovelli et al., 2010). We demonstrated that the simultaneous inhibition of Bcl-2 and mTOR pathways results in higher apoptotic effects and decreased proliferation in ALL cell lines and primary cells. Nevertheless, the evidence of a phenotype resistant to the ABT-737/CCI-779 combination prompts further studies on the simultaneous interruption of multiple pathways in ALL.

3.2 RAS/RAF/MEK/ERK pathway

In the last decades, the RAS/RAF/MEK/ERK pathway (Fig. 1) has emerged as one of the key components of the signaling network that regulates several cellular processes, such as cell growth, proliferation, survival, and, under certain conditions, also differentiation, migration and apoptosis (Tortora et al., 2007; Steelman et al., 2011). The RAS cascade is activated by a large number of extracellular stimuli and various internal processes. At the intracellular level, through a sequential phosphorylation and activation of protein kinases, regulates a wide array of substrates, including transcription factors and other protein kinases (Steelman et al., 2011; Wang et al., 2007).

Deregulation of RAS signaling is commonly found in cancer cells and is often caused by mutations in the RAS family of genes or by endogenous receptor-ligand interactions, resulting in a constitutive activation of G proteins. Mutations in NRAS and KRAS2 have been demonstrated in 15% and 22%, respectively, of childhood ALL (Case et al., 2008). A

persistently active form of RAS signaling can also arise from mutations in genes encoding others proteins that impinge on this signaling pathway and that have recently been shown to be mutated in childhood ALL, such as FLT3, PTPN11, and BRAF (Taketani et al., 2004; Tartaglia et al., 2004). By a complete mutational screen of key exons of NRAS, KRAS2, PTPN11, FLT3, and BRAF in a large cohort of unselected ALL cases at diagnosis and at relapse, Case et al. (2008) showed that somatic mutations of these genes represent a common genetic aberration in childhood ALL and are related to disease progression, thus providing the rational base for including RAS inhibitors as novel targeted therapy for childhood ALL. Evidences provided by our group and others, indicate that the RAS/RAF/MEK/ERK pathway is an extremely promising therapeutic target in hematological malignancies. Studies performed in ALL have shown constitutive ERK phosphorylation in 30% of clinical samples and higher ERK kinase activity in ALL primary cells, compared to normal BM cells (Meng et al., 2003; Towatari et al., 1997). During the past years, we have described the incidence and prognostic impact of constitutive ERK activation in a large series of ALL clinical samples taken at diagnosis from 131 patients uniformly treated according to the GIMEMA LAL 2000 study protocol (Gregorj et al., 2007). One third (34,5%) of these samples showed constitutive activation of ERK, as evaluated using a flow cytometric assay that quantifies phosphoprotein expression in individual cells. We found that constitutive ERK activation was associated with higher WBC counts (p=0.013); most importantly, ERK phosphorylation was an independent predictor of failure to achieve CR (P=0.027) in multivariate analysis. Consequently, strategies aimed at inhibiting MEK activity have been investigated experimentally.

Potent small-molecules that selectively inhibit different molecular targets of the RAS/RAF/MEK/ERK pathway have been developed (Wang et al., 2007). These compounds have shown promising anticancer activity *in vitro* and *in vivo* by suppressing tumor growth and/or inducing apoptosis in a broad spectrum of solid tumors and in hematological malignancies including AML, multiple myeloma (MM), lymphomas and ALL. Some of them are currently under investigation in early-phase clinical trials (Chappell et al., 2011).

We have investigated the molecular and functional consequences of the pharmacological disruption of the MEK/ERK module by selective inhibitors of MEK. However, discouraging preliminary results were observed in both ALL primary cells and cell lines exposed *in vitro* to the MEK inhibitors PD98059 and PD0325901, with significant inhibition of ERK phosphorylation in a proportion of samples, but neither occurrence of cell cycle changes nor induction of apoptosis (Tafuri et al, unpublished results). These data may suggest that in ALL pro-survival signals could be mediated by additional pathways. Nevertheless, since one of the most exciting features of MEK inhibitors as potential anticancer agents is their ability to lower cancer cells' apoptotic threshold, sensitizing tumor cells to the proapoptotic action of other agents, they represent useful tools for building up pharmacological combinations with synergistic proapoptotic effects (Milella et al., 2005; Tortora et al., 2007).

4. Apoptosis modulators

In order to maintain cells homeostasis, a correct balance between cell proliferation and survival is preserved by several mechanisms, including the physiologic cell death program, called "apoptosis". During this process, old, mutated or irreparably damaged cells are removed from the organism in a properly regulated fashion, without eliciting an

inflammatory response (Danial & Korsmeyer, 2004). In mammalian cells, apoptosis can be triggered by a broad variety of stimuli such as developmental signals, cellular stress or DNA damage, cytotoxic insults or environmental factors, resulting in activation of two different apoptotic signaling pathways: the extrinsic and the intrinsic pathway. The extrinsic pathway is activated in response to the binding of extracellular signals to cell surface death receptors (Ashkenazi et al., 1999) and it is independent of mitochondrial involvement. Instead, the intrinsic pathway is activated by intracellular events that involve the release of apoptotic factors from mitochondria (Danial & Korsmeyer, 2004). In particular, the intrinsic pathway involves a mechanism called "mitochondrial outer membrane permeabilization" (MOMP) by which several apoptotic factors such as cytochrome c, AIF and Smac-DIABLO, are released into the cytoplasm (Green & Reed, 1998; Wang et. al, 2001). MOMP following cell-intrinsic apoptotic stimuli is to a large extent regulated by the Bcl-2 family of proteins (van Loo et al., 2002), which thus constitute a critical checkpoint of the intrinsic pathway. Bcl-2 was first identified at the chromosomal breakpoint of t(14;18)-bearing human follicular B-cell lymphoma (Tsujimoto et al., 1985) and its oncogenic potential has been demonstrated through a gene transfer approach (Reed et al., 1988). The Bcl-2 family is an evolutionarily-conserved family of proteins that consists of more than 20 members which can be divided into three subgroups based on their function and on the presence of conserved Bcl-2 homology (BH) regions (Fig. 2). The most important members of the Bcl-2 antiapoptotic repertoire contain four Bcl-2 homology domains (BH1–4) and are generally integrated within the outer mitochondrial membrane (OMM), including Bcl-2, Bcl-2 related gene A1 (A1) and the long isoform Bcl-xL, Bcl-w and myeloid cell leukemia 1 (Mcl-1). Their function is to preserve and maintain OMM integrity by direct inhibition of the proapoptotic members of the family (Green & Evan, 2002). The proapoptotic proteins are divided into two groups: effector proteins and BH3-only proteins. The multi-region proapoptotic effector proteins, Bax and Bak, were conventionally thought to share BH 1–3 regions; however, by structure-based alignment of globular Bcl-2 family proteins, a conserved BH4 motif was recently observed (Kvansakul et al., 2008). Following activation, Bak and Bax undergo homo-oligomerization thus promoting MOMP by formation of proteolipid pores within the OMM. The BH3-only proteins, sharing the homology with Bcl-2 in the BH3 region only, are divided based on their ability to interact either with the antiapoptotic members or with both the antiapoptotic and the effector proteins. BH3-only proteins such as Bad (Bcl-2 antagonist of cell death) and Noxa are referred to as ''sensitizer'' and/or ''derepressor" and interact, through the BH3 region, only with the antiapoptotic members of the family. The BH3-only proteins that are referred as ''direct activators'', such as Bid (Bcl-2-interacting domain death agonist) and BIM (Bcl-2-interacting mediator of cell death), can interact with the antiapoptotic repertoire as well as with the effectors, and can directly induce Bak and Bax oligomerization and MOMP (Chipuk et. al, 2010). Correct interactions between pro and antiapoptotic Bcl-2 family members are crucial for the normal activation of the intrinsic pathway and apoptosis regulation.

Resistance to apoptosis, frequently caused by overexpression of antiapoptotic proteins, is a common feature of cancer. In fact, it has been demonstrated that deficiency in apoptosis is one of the key hallmarks of cancer (Hanahan & Weinberg, 2000). High levels of Bcl-2 expression have been found in many cancers, such as follicular lymphoma (Gaulard et al., 1992), chronic lymphocytic leukemia (CLL) (Schena et al., 1992), AML (Andreeff et al., 1999), MM (Harada et al., 1998), small cell lung cancer (Ben-Ezra et al., 1994) and melanoma (Leiter et al., 2000). Elevated expression of the antiapoptotic proteins Bcl-2, Bcl-xL and Mcl-1 has

been reported also in ALL cell lines and primary samples (Campana et al., 1993; Coustan-Smith et al. 1996; Del Gaizo Moore et al., 2008; Hogarth & Hall, 1999). In particular, several studies have shown that higher Bcl-2 levels are associated with improved ability of ALL cells to survive under very unfavorable culture conditions, such as the absence of stromal-derived growth factors. Therefore, Bcl-2 overexpression may contribute to leukemogenesis and influences the survival ability of leukemic lymphoblasts. Moreover, Bcl-2 overexpression may provide an explanation of the ability of leukemic lymphoblasts to expand outside the bone marrow microenvironment (Campana et al., 1993; Coustan-Smith et al., 1996). To test the hypothesis that the apoptotic defects are essential for tumor maintenance, Letai et al. (2004) have generated a transgenic mouse model expressing a conditional Bcl-2 gene and constitutive c-myc that develop lymphoblastic leukemia. They have shown that Bcl-2 elimination yielded rapid loss of leukemic cells and significantly prolonged mouse survival, formally validating Bcl-2 as a rational target for a targeted cancer therapy (Letai et al., 2004). Furthermore, high Bcl-2 levels are observed also in ALL characterized by t(4;11) (Robinson et al., 2008). It has been demonstrated that the equilibrium in the formation of Bcl-2:Bax heterodimers (suppressors of death) and Bax:Bax homodimers (activators of death) appears to be central in the molecular regulation of apoptosis (Coustan-Smith et al, 1996). In fact, high Bax expression alone was shown to be associated with an increased probability of relapse (Hogarth & Hall, 1999) and both Bax expression levels and the Bax/Bcl-2 ratio are significantly lower in samples at relapse, as compared with samples at initial diagnosis (Prokop et al., 2000). Kaufman et al. (1998) have shown that Mcl-1 is highly expressed in ALL at relapse, resulting two times higher as compared to pre-treatment levels. Further studies have correlated increased expression of Bcl-2 antiapoptotic family members to increased tumor cell survival and drug resistance *in vitro* and *in vivo* (Del Gaizo Moore et al., 2008). Holleman et al. (2004) observed overexpression of the antiapoptosis gene Mcl-1 in prednisolone-resistant ALL. Wei et al. (2006) have shown that Mcl-1 is also involved in glucocortocoid (GC) resistance in ALL. In this report, by combining bioinformatic analyses of gene expression profiles of childood ALL (classified as GC-sensitive/resistant by *ex vivo* testing) with functional data obtained from experimental systems, they identified Mcl-1 as the key member of the antiapoptotic Bcl-2 family, responsible for GC resistance (Wei et al., 2006). These observations suggest that inhibition of the antiapoptotic/pro-survival members of Bcl-2 family proteins may represent an attractive approach for the treatment of ALL.

Several strategies have been developed to inhibit the activity of the Bcl-2 family of proteins, including antisense oligonucleotides, peptides derived from the BH3 domain, and small molecule antagonists. While clinical studies with peptides rarely progress to late-stage trials, because of poor pharmacological properties (Lessene et al., 2008), small molecules proved more effective. Small molecule inhibitors are designed to compete with activator and sensitizer molecules for the same binding site on Bcl-2 family members (the BH3 domain) and consequently they are referred to as BH3 mimetics (Richardson & Kaye, 2008). Several molecules has been developed as BH3 mimetics; one the most attractive is ABT-737 (Oltersdorf et al. 2005) and its orally bioactive analog ABT-263. Apoptosis induced by ABT-737 is dependent on Bax and Bak (Van Delft et al., 2006) suggesting that its primary mechanism of action is through the regulation of the intrinsic apoptosis pathway. ABT-737 is a Bcl-2/Bcl-xL inhibitor that specifically binds the hydrophobic groove that normally serves as a binding site for the BH3 domain of activators and sensitizers. ABT-737 binds

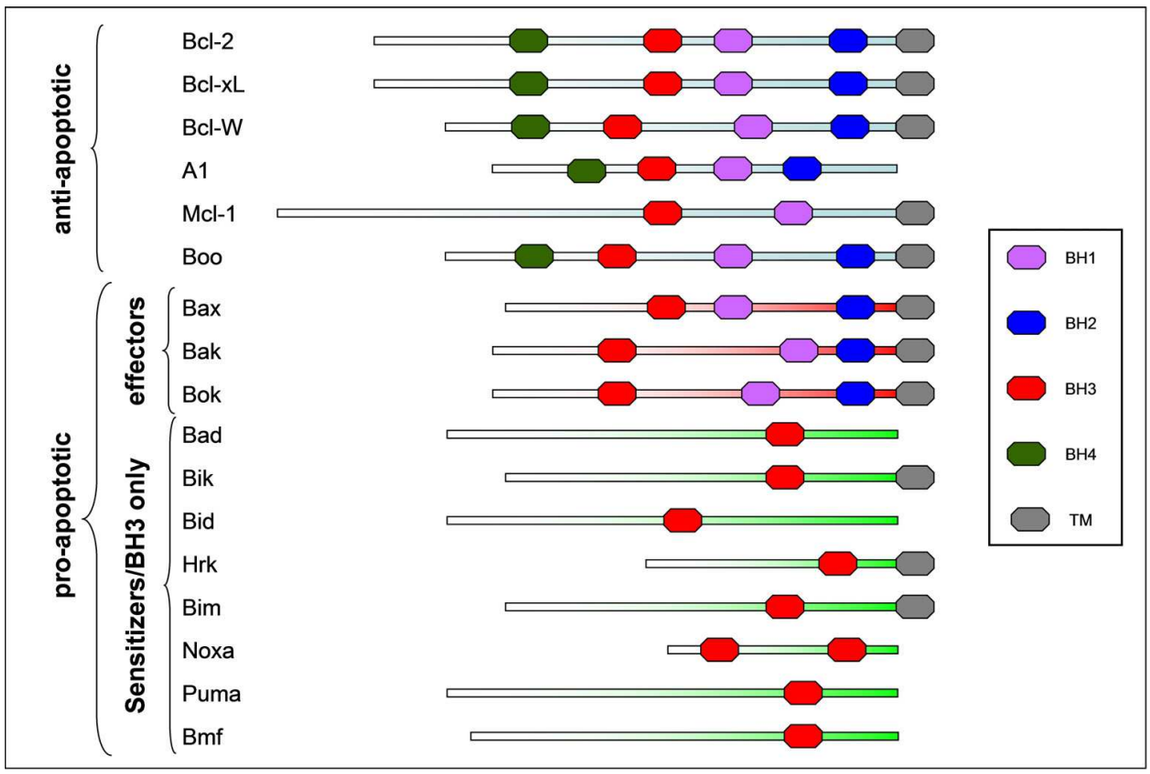

Fig. 2. The Bcl-2 family of proteins (TM: transmembrane domain).

potently Bcl-2, Bcl-xL and Bcl-w, instead only weakly Bfl/A1 or Mcl-1. In fact, Mcl-1 overexpression is reported to be one of the principal factors that may confer resistance to ABT-737 (Konopleva et al., 2006). Pre-clinical studies have shown that ABT-737 displays a potent growth-inhibitory activity in ALL. In particular we and others have evaluated preclinical activity of ABT-737 on a panel of ALL cell lines and primary cells obtained from ALL patients, observing a strong increase in apoptosis induction (Del Gaizo Moore et al., 2008; Tafuri et al., 2007). Furthermore, we employed a combined preclinical strategy to overcome the ABT-737 resistance in ALL cells using the mTOR inhibitor CCI-779. We observed synergistic effects on apoptosis induction between ABT-737 and CCI-779 in both ALL cell lines and in a proportion of primary cells (Iacovelli et al., 2010). Other studies employing ABT-737 in combination with L-asparaginase, topotecan, vincristine and etoposide have shown increased antileukemic effects against drug-resistant ALL xenografts models (High et al., 2010). Since ABT-737 was not orally bio-available, the oral analog ABT-263, capable of initiate apoptosis following 2 hours from administration, has been developed. The dosing flexibility of ABT-263 accomplished by its oral efficacy prompt the clinical investigation of this molecule, both as a single agent and in combination. The ABT-263 is currently employed in phase I clinical trials in both hematological and solid tumors (Gandhi et al., 2011; Wilson et al., 2010). GX15-070 (Obatoclax) is another BH3 mimetic, which binds to Bcl-2, Bcl-xL and Mcl-1 with comparable affinity (Lessene et al., 2008); for this reason, Obatoclax may potentially overcome resistance conferred by high levels of Mcl-1, inhibiting the interaction between Bak and Mcl-1 (Trudel et al., 2007). Several reports have shown that increased Mcl-1 expression is associated with GC resistance (Holleman et al., 2004; Stam et al., 2010) and it has been demonstrated that Obatoclax treatment can re-

sensitizes GC-resistant ALL cells (Bonapace et al., 2010). Other results suggest that Obatoclax could be useful as a therapeutic agent against ALL and that the activity and/or the expression of antiapoptotic proteins could be used as a biomarker to determine the appropriate treatment strategy for ALL patients (Heidari et al., 2010). In particular, Bonapace et al have shown that Obatoclax can overcome the resistance to GC through rapid activation of autophagy-dependent necroptosis, which was able to bypass the apoptotic block in the mitochondrion. This effect required Obatoclax-mediated dissociation of Beclin-1 from Mcl-1 and was associated with inhibition of mTOR activity (Bonapace et al., 2010). Autophagy is a cell death mechanism distinct from apoptosis, also called type 2, non-apoptotic cell death. Autophagy is characterized by the inclusion of cytoplasmic material into vacuoles, leading to degradation mediated by lysosomal enzymes (Klionsky, 2007). Beclin-1 is a novel BH3 domain protein involved in autophagy, which plays an essential role in the activation of this cell death process. Beclin-1 can be activated or inactivated by its interaction with the pro or antiapoptotic members of the Bcl-2 family, respectively, through the BH3 domain. Thus, the cells can regulate autophagy by targeting Beclin-1 through Bcl-2 family members (Levine et al., 2008). Small molecules, such as ABT-737 and Obatoclax, by acting as BH3 inhibitors, can overcome resistance mediated by Bcl-2 antiapoptotic members, thereby restoring the cellular ability to induce autophagy (Bonapace et al., 2010; Levine et al., 2008).

The use of the BH3 mimetics, alone or in combination with other inhibitors or chemotherapy, can be of great importance in customizing an individual patient's therapy especially in cases in which chemotherapy alone is not able to decrease tumor burden.

5. Epigenetic silencing

The term "epigenetic" is generally referred to heritable changes in gene expression not caused by alterations in the DNA coding sequences. Epigenetic changes such as DNA methylation or histone modifications work in concert with each other to regulate gene expression in normal mammalian development (Baylin & Ohm 2006). However, it is now evident that epigenetic modifications considerably contribute to development and progression of carcinogenesis in general, and of leukemogenesis in particular (Baylin & Ohm, 2006; Chen et al., 2010).

5.1 DNA methylation

DNA methylation is mediated by a family of enzymes, DNA methyltransferases (DNMTs), which catalyze the covalent addition of a methyl group at the 5′ carbon of cytosine residues that precede guanosine (CpG) islands (Herman & Baylin, 2003). The resulting 5-methylcytosines protrude into the major groove of DNA, inhibiting gene transcription.

In mammalian cells, 5-methylcytosine is found in approximately 5% of the whole genome. Compared to normal cells, cancer cells display aberrant methylation of cytosine residues both in gene promoters or coding regions, leading to a transcriptional silencing of tumor suppressor gene. Cyclin-dependent kinase inhibitors, such as CDKN2B, which encodes the tumor suppressor p15 (INK4B), and CDKN2A, which encodes the tumor suppressors p16 (INK4A) are, among other genes, frequently methylated in their promoter regions (Garcia-Manero et al., 2009; Roman-Gomez et al., 2004). This epigenetic modification has been described also in ALL. Results obtained by Roman-Gomez at al. (2004) in 251 consecutive ALL patients demonstrated that promoter hypermethylation of multiple genes is a common

phenomenon in ALL and is a strong independent prognostic factor in predicting the clinical outcome of ALL patients. In particular, they observed that methylation in ALL cells mostly participate to inactivation of genes involved in: 1) cell growth regulation by controlling directly (p15, p16 and p57) or indirectly (p73, PTEN, NES-1) cell cycle check-points; 2) apoptosis inhibition (p14, TMS1, APAF-1 and DAPK); 3) cell adhesion (some members of cadherin family). However, the relationship between aberrant DNA methylation and protein expression of tumor suppression genes has not yet been extensively evaluated in adult ALL series. Bueso-Ramos et al. (2005) showed that methylation of more than one gene of the pathway composed of p73, p15 and p57 was associated with a worse outcome of adult ALL patients. However, lack of association was reported between p73 protein expression and clinical-biologic characteristic. We analyzed the promoter methylation status of p73, p21, p15 and p16, evaluating in addition the p21, p15 and p16 protein expression, in primary cells from newly diagnosed adult ALL patients, uniformly treated according to the GIMEMA LAL2000 protocol. Our results indicate that in vivo p15 and p21 protein expression plays an unfavorable prognostic role in adult ALL patients independently of the p73, p21, p15 and p16 gene promoter methylation status (De Cave et al. 2007). Data reported by Yang et al. (2009) demonstrated that detection of epigenetic alterations allows the identification of ALL patients with poor prognosis within the standard-risk group. More recently, Milani L et al. (2010), analyzing the methylation patterns of CpG sites in 416 genes, have found a striking difference in the methylation patterns within a large number of samples from ALL patients. Notably, they observed a correlation between the methylation level and clinical outcome within major subgroups of ALL patients, identifying 20 genes with DNA methylation levels capable to predict leukemia relapse. These observations suggest that methylation analysis should be explored to identify ALL patients at different risk.

5.2 Histone modifications

Structural studies have demonstrated that histones easily undergo post-translational modifications in their long amino-teminal tails that project outward from the nucleosome. These tail modifications, which include acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation, are involved in several biological processes related to the chromatin structure, i.e. gene regulation, DNA repair, DNA replication, chromatin condensation etc. (Bhaumik et al., 2007).

Histone acetylation acts in concert with DNA methylation to regulate gene expression. Several data have demonstrated that histone acetylation, catalysed by histone acetyltransferases (HATs), is involved in maintaining chromatin structure in a transcriptionally active form (Chen et al., 2010). In contrast, histone deacetylation (HDAC), removing acetyl groups from histone tails, permits the histones to wrap more tightly around the DNA and thus maintains genes inactivated and silenced. High level of HDAC expression is a common finding in cancer cells (Moreno et al, 2010) and accounts for the aberrant expression and activity of numerous proteins involved in proliferation, differentiation, apoptosis, adhesion and migration (Lane & Chabner, 2009). A high expression of HDAC has also been described in hematological malignancies, particularly in lymphomas (Marquard et al, 2009) and in AML (Cimino et al, 2006). Thus far, very few data are available about HDAC expression in ALL.

In a recent study conducted by Moreno and colleagues (2010) on childhood ALL samples, the authors observed a differential HDACs expression between clinical samples.

Particularly, they found a higher expression of HDAC1 and HDAC4 and a lower expression of HDAC5 in T-ALL. Moreover, they reported that higher expression of HDAC7 and HDAC9 is associated with poor prognosis both in the overall group of childhood ALL and in B-lineage CD10-positive cases suggesting the use of HDAC inhibitors as a promising therapeutic intervention for the treatment of refractory childhood ALL.

5.3 Epigenetic therapy

Because of the key role of epigenetic modifications in the pathogenesis of cancer, pharmacological agents that target components of the epigenetic machinery are become promising elements of the therapeutic arsenal for cancer treatment.

Several data have indicated that DNA methyltransferase inhibitors (DNMTi), such as azacitidine, decitabine, and other derivative, are able to restore tumor suppressor gene expression and exert antitumor effects *in vitro* and *in vivo* by inhibiting hypermethylation (Chen et al., 2010). Clinical studies have clearly demonstrated that DNMTi, used alone or in combination, may convey clinical benefit to patients with hematological malignancies, especially myelodysplastic syndrome and AML. In contrast, the application of this therapeutic strategy to ALL patients is so far limited. Recent data reported by Schafer et al. (2010) have demonstrated that MLL rearranged (MLL-r) infant ALL samples, compared with other childhood leukemias and normal controls, are characterized by promoter hypermethylation of several genes. Notably, they showed that decitabine preferentially kills MLL-r lymphoblastic leukemia cell lines and also that this response correlates with the upregulation of several of the identified silenced genes, suggesting predictable efficacy of demethylating agents in this category of infant ALL that is reported as the most aggressive type of childhood leukemia. More recently, studying the same category of MLL-r infant ALL patients, Stumpel et al. (2011) observed that in samples from infant ALL patients carrying the t(4;11), eleven miRNAs were downregulated as a consequence of hypermethylation and seven of these were re-activated after exposure to a demethylating agent, thus providing additional evidence that demethylating agents should be tested for their efficacy in MLL-r infant ALL patients.

HDAC inhibitors (HDAC-Is) are a class of agents with the capacity to induce acetylation of histone and non-histone proteins (Lee et al. 2010). These molecules have been intensively investigated in preclinical models as well as in clinical trials for a variety of malignancies, because of their ability to inhibit proliferation, induce differentiation, and cause apoptosis in tumor cells (Bolden et al. 2006; Lee et al., 2010). Since only a portion of patients has a therapeutic response, a very important issue is the need to identify markers of potential response or resistance to HDAC-Is. Currently, there is great interest in the HDAC-Is field, as several new and more effective compounds are being developed and entering clinical trials. In ALL, HDAC-Is have been used only for *in vitro* studies. It has been demonstrated that HDAC-Is induce apoptosis in ALL cell lines (Moreno et al., 2010; Romanski et al., 2004), including those resistant to glucocorticoid, and in *ex vivo*-cultured samples. Our preliminary results *in vitro* show that novel HDAC-Is are potent growth inhibitors and inducers of apoptosis in human leukemia cells, including ALL cell lines, and suggest their potential therapeutic use for patients with leukemias.

Future research will definitely suggest additional therapeutic targets regulating epigenetic pathways and continued clinical trials with demethylating agents and HDAC inhibitors, alone or in combination, will undoubtedly provide further advance in the treatment of hematological malignancies, including ALL cases.

6. Bone marrow microenvironment

Bone marrow (BM) is a complex and dynamic network of hematopoietic and stromal cells, blood vessels, proteins and others microenvironmental factors, such as growth factors and cytokines that compose the extracellular matrix (ECM) (Ayala et al., 2009). The interaction between hematopoietic and stromal or mesenchymal cells, as well as between cellular and soluble components of BM microenvironment, sustains normal hematopoiesis by promoting cell growth, regulating differentiation, and importantly maintaining a pool of undifferentiated pluripotent and long-lived stem cells (Ayala et al., 2009; Rizo et al., 2006). The BM microenvironment is critical for B-cell lymphopoiesis.

Similar to normal hematopoiesis, the survival and growth of leukemic cells largely depend on the support provided by the BM microenvironment, which also contributes to blunt the effects of chemotherapy and provides a sanctuary for minimal residual disease. Notably, several data have demonstrated the key role of BM microenvironment in supporting the survival of leukemic stem cells (LCS), also known as "leukemia initiating cells", i.e. the subpopulation of cells within a tumor that are long lived, has the potential to self-renew, can generate the original tumor in xenograft models (Konopleva & Jordan, 2011; Moore & Lyle, 2011) and are therefore thought to be the cause of relapse in patients treated with traditional drugs. The emerging concept of LSC niche as a dynamic entity in which interactions between the LCS and its microenvironment take place are now becoming a hot topic highlighting the relevance of BM microenvironment for leukemia initiation, progression and drug resistance and opening up opportunities for therapeutic intervention (Ayala et al., 2009; Rizo et al., 2006).

The mechanism by which the BM stroma modulates growth, progression and response to chemotherapy of ALL cells is not fully elucidated. In vitro studies of primary ALL cells usually demonstrate a large amount of spontaneous ALL cell apoptosis which could be reduced by co-culturing them with BM stromal cells, increasing survival and proliferation and blunting the cytotoxic effects of chemotherapeutic agents (Brown et al., 2008). There are evidences that interactions of ALL cells with BM stroma are mediated by different members of the integrin family of proteins, constitutively expressed on leukemic cells (LFA-1 on T-ALL and VLA-4 on B-ALL), with their respective ligands exposed on the surface of BM stromal cells or secreted in the ECM (fibronectin) (Ayala et al., 2009; Tabe et al., 2007). These interactions lead to the activation of tyrosine kinases, and consequently of downstream pathways which regulate cell growth, survival, adhesion, migration, angiogenesis, apoptosis and autophagy (Tabe et al., 2007; Veiga et al., 2006). The PI3K/PTEN/AKT/mTOR cascade is a critical pathway in stromal/leukemic cells interactions mediated by integrins, chemokines (such as CXCR4/CXCL12), angiogenic factors and interleukins (Konopleva & Jordan, 2011).

It is well known that interleukins, such as IL-7 and IL-3, have a critical role in regulation proliferation and survival of T- (Scupoli et al., 2007) and B-ALL (Juarez et al., 2007) cells by activating stroma interactions. Addition of IL-7 to co-cultures of BM stromal cells and ALL cells has been reported to increase ALL cell survival through phosphorylation of AKT, ERK1/2, and p38 (Juarez et al., 2007). Crosstalk between BM microenvironment and leukemic cells has also been demonstrated by upregulation of IL-8 in leukemic cells promoted by CXCR4/CXCL12-induced activation of the NF-kB and JNK/AP-1 pathways (Scupoli et al., 2008). High levels of IL-8 could in turn enhance angiogenesis in the BM microenvironment and indirectly sustain tumor growth (Scupoli et al., 2008). Ultimately, it

has been demonstrated that CXCR4 has a role in homing and migration of ALL blasts in BM and in other organs (Konopleva & Jordan, 2011; Sipkins et al., 2005).
Increased neovascularization, which effectively contributes to growth and progression of cancer, has been described in ALL as a result of autocrine and/or paracrine loops of the main proangiogenic mediators (bFGF, VEGF and angiopoietin) promoted by BM stroma/leukemic blast interactions (Ayala et al., 2009). Beside marked neovascularisation, extensive endothelial cell proliferation represents one of the most pronounced microenvironmental changes observed in BM of ALL patients (Veiga et al., 2006). Several studies have demonstrated the existence of a crosstalk between the endothelium and leukemic cells, in which leukemia cells stimulate BM endothelium and promote de novo angiogenesis and neovascularisation, while BM endothelium promoted leukemia cell survival through modulation of antiapoptotic bcl-2 family members (Veiga et al., 2006). Another study by Aref et al. (2007) described in B-ALL an abnormal expression of matrix metalloproteinases (MMP), which is a further class of angiogenesis and tumor progression regulators produced by both stromal and leukemic cells (Aref et al., 2007)
A further interesting aspect related to BM microenvironment and leukemia cells interactions regards the role of BM microenvironment on lineage commitment and differentiation. Several studies have indeed demonstrated that BM microenvironment can drive lineage-specific differentiation in leukemia by modulating different cytokines and other soluble components (Kankuri et al., 2008; Wei et al., 2008). In an elegant study performed on a human MLL-AF9 leukemia model transplanted into immunodeficient mice, Wei J et al. (2008) showed that ALL, AML or mixed-lineage leukemia were generated by altering the cytokine milieu of human CD34+ cells expressing MLL-AF9, thus demonstrating the importance of the microenvironment in driving leukemia phenotype.
Finally, a translationally relevant concept is that the BM microenvironment can also regulate the response of leukemic cells to chemotherapy through a variety of mechanisms. According to the evidences described before, BM microenvironment mainly contributes to protection of leukemic cells from drug effects participating in induction of a chemo-resistant phenotype. For instance, high levels of asparagine synthetase produced by BM-derived mesenchymal cells have been demonstrated as one of the mechanisms participating in the resistance to asparaginase in ALL samples (Iwamoto et al., 2007). Moreover, it has been reported that IL-7 and TSLP protect ALL cells in vitro from MTI-induced effects suggesting that stromal cell-derived cytokines can contribute to MTI resistance (Brown et al, 2007).
In conclusion, these evidences demonstrate the importance of interaction between BM stromal cells and ALL blasts and chemoresistance, the role of BM microenvironment and ultimately the potential of therapeutic approaches based on disrupting this interaction in ALL patients.

7. From personalized proteomic profiles to personalized medicine in ALL

In order to tailor a personalized therapy, it is mandatory that research is aimed at identifying specific targets on the malignant cells in order to dissect groups of patients with different risks and to develop new drugs with selective activity against the tumor cell. The characterization of molecular profiles of cancer cells, compared to their normal counterparts, requires the continuous development of comprehensive molecular analysis technologies. It is particularly crucial to implement and support the development of novel technologies that

allow high-throughput analysis of genetic alterations, expression of genome products and monitoring the STP in cancers. Although a complete description of these new technologies is beyond the scope of this chapter, some observations regarding the most promising techniques that may allow to further decipher the molecular profile of leukemia samples at the post-translational level are herein reported.

Understanding cancer cell physiopathology at the protein level requires the development of reliable proteomics. In addition, profiling and classification of several components of multiple aberrant STP would be expected to accurately predict disease behavior and prognosis. Moreover, the challenge of translating proteomic pathway profiling to the bedside would require a technique capable of efficiently processing small numbers of cancer cells by routinely assessing multiple STP simultaneously. Traditional protein assay techniques like Western blotting (WB) and ELISA assay can assess the level of protein phosphorylation. WB can evaluate a limited number of proteins and samples in a semi-quantitative way requiring large amounts of cellular material. The ELISA assay gives quantitative information but is extremely expensive. Further attempts have been made by MALDI-TOF (matrix assisted laser desorption/ionization-time of flight) and by two-dimensional gel-electrophoresis. However, both methods cannot be performed on a large scale on clinical samples and, more importantly, they require large numbers of cells (Petricoin et al., 2005)

The Reverse Phase Protein Arrays (RPPA) is an emerging, sensitive, high-throughput technology that allows profiling the STP working state of large clinical populations, examining the functional proteomic profile of each sample. By quantitative analysis of minuscule amounts of proteins (nanoliters of protein lysates equivalent to picograms of proteins), RPPA permits the measurements of proteins and their corresponding phosphoproteins by using high-quality monoclonal antibodies. At the same time, cell lysates collected from hundred of samples are spotted on a single slide, making this methods highly effective in retrospective clinical sample analysis or in prospectively collected sets of samples. Compared to WB, which uses proteins from 5×10^5 cells for each antibody analysis, RPPA requires proteins from only 5.000-20.000 cells per sample preparation and a protein amount equivalent to 200 cells is spotted per slide for a single antibody. Therefore, 20.000 cells would be sufficient to analyze 100 different protein targets and the amount of material previously used for single WB will be now used for 2500 antibodies by RPPA. This will thus be particularly useful for protein detection of rare populations (stem cells, residual cells surviving after chemotherapy) (Kornblau et al., 2009). RPPA has been successfully applied in a number of basic and clinical studies (Accordi et al., 2010; Kornblau et al., 2009; Petricoin et al., 2007). In a very recent study published by Accordi et al. (2011) the RPPA technique was applied to analyze the activation/expression status of 92 key signaling proteins in 118 pediatric B-ALL patients. The authors found an aberrant activation and/or increased expression of several pathways involved in cell proliferation (such as Bcl-2 hyperphosphorylation, LCK and cyclin E up-modulation) in patients with poor prognosis. Our group has applied RPPA on adult and pediatric ALL patients, identifying B- and T-lineage ALL by the proteomic profile, showing in addition a prognostic role of cell cycle and apoptotic molecule over-expression in ALL patients (Tafuri et al. unpublished results).

Alternatively to the phospho-proteomic array technology, it is possible to perform a "single-cell proteomic" analysis by using flow cytometry (Irish et al., 2006). The powerful multi-parametric single cell analysis platforms nowadays available allow simultaneously determining and quantifying the active or inactive state of multiple signaling components in

individual cancer cells using fluorophore-conjugated antibodies. A "single-cell signaling signature" can be obtained for each cell in a mixed population and compared either with other features on the same cell or with normal cells present in patient samples. Therefore, single-cell analysis by flow cytometry is particularly useful to analyze samples for rare (HCS or LCS) and/or heterogeneous cell type populations. Recent data published by Gibbs et al (2011) demonstrated the biochemical and functional heterogeneity of human HSCs by using single-cell phospho-specific flow cytometric analysis. Analyzing the response profile of human HSCs to a broad range of hematopoietic cytokines the authors demonstrated that the HSC compartment is composed of biochemically distinct subsets, and that cellular proliferation can be directly regulated by G-CSF.

8. Concluding remarks

Comprehensive molecular analysis of cancer over the past decades has clearly shown that most malignancies are composed of several molecular subgroups, each defined by specific genetic/epigenetic alterations and with different prognosis. In the past years, diagnostic and prognostic achievements obtained through molecular genetics have improved patient follow-up, addressing treatment choice and anticipating treatment failure. In the post-genomic era, the translational approach to diseases, especially to hematologic and solid malignancies, must exceed including new tools for dissecting the functional proteomic and metabolomic profiling. Future clinical technologies may expand studies on protein arrays helping to define in large clinical population the functional proteomic profile at steady-state; this approach may lead to the identification of aberrant molecular target(s), possibly guiding the choice of specific molecular therapies, on one hand, and leading to elucidate of post-treatment changes, on the other hand, with the overall objective of improving the strategies currently employed to counteract leukemia chemo- and targeted drug-resistance. The ongoing new challenge will be to manage the enormous amount of data often produced by these technologies, approaching statistical methods that may accurately identify the most important clinical and biological variables.

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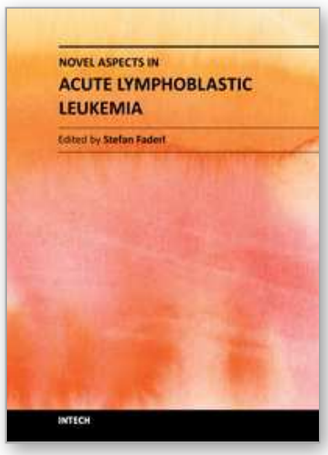

Novel Aspects in Acute Lymphoblastic Leukemia
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Acute lymphoblastic leukemia (ALL) has turned from a universally fatal to a highly curable disease in little more than four decades. Even though differences in outcome continue to exist between children and adults, intense efforts are under way to overcome this discrepancy and improve the prognosis of adult patients as well. This exemplary progress in ALL therapy has been possible by the combination of an increasingly better understanding of the biology of the disease, availability of a range of effective drugs, and astute designs and relentless executions of many clinical trials. ALL is a complex disease requiring complex therapy. Whereas this book cannot provide a comprehensive review of every one of its many facets, the chapters from many investigators from around the world nevertheless cover a number of relevant topics: aspects of the epidemiology of ALL in Hispanics, ophthalmologic manifestations of ALL, overviews of current therapy and drug-resistance mechanisms, novel biological pathways and targets, new drugs in development, and long-term consequences of CNS prophylaxis and therapy. The publishers and editor therefore hope that the prospective readers will find enough insight and information for their own endeavors.

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