# Histamine $H_2$ Receptor in Blood Cells: A Suitable Target for the Treatment of Acute Myeloid Leukemia

Federico Monczor, Sabrina Copsel, Natalia Fernandez, Carlos Davio, and Carina Shayo

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**Abstract**

Acute myeloid leukemia (AML) consists in a cancer of early hematopoietic cells arising in the bone marrow, most often of those cells that would turn into white blood cells (except lymphocytes). Chemotherapy is the treatment of choice for AML but one of the major complications is that current drugs are highly toxic and poorly tolerated. In general, treatment for AML consists of induction chemotherapy and post-remission therapy. If no further post-remission is given, almost all patients will eventually relapse. Histamine, acting at histamine type-2 ($H_2$) receptors on phagocytes and AML blast cells, helps prevent the

F. Monczor (✉) • N. Fernandez • C. Davio
Instituto de Investigaciones Farmacológicas, ININFA, Universidad de Buenos Aires—Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Junín 956 PP, (1113), Buenos Aires, Argentina
e-mail: monczorf@ffyb.uba.ar

S. Copsel
Microbiology and Immunology Department, Miller School of Medicine, University of Miami, Miami, FL, USA

C. Shayo
Laboratorio de Patología y Farmacología Molecular, Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina


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production and release of oxygen-free radicals, thereby protecting NK and cytotoxic T cells. This protection allows immune-stimulating agents, such as interleukin-2 (IL-2), to activate cytotoxic cells more effectively, enhancing the killing of tumor cells. Based on this mechanism, post-remission therapy with histamine and IL-2 was found to significantly prevent relapse of AML. Alternatively, another potentially less toxic approach to treat AML employs drugs to induce differentiation of malignant cells. It is based on the assumption that many neoplastic cell types exhibit reversible defects in differentiation, which upon appropriate treatment results in tumor reprogramming and the induction of terminal differentiation. There are promissory results showing that an elevated and sustained signaling through $H_2$ receptors is able to differentiate leukemia-derived cell lines, opening the door for the use of $H_2$ agonists for specific differentiation therapies. In both situations, histamine acting through $H_2$ receptors constitutes an eligible treatment to induce leukemic cell differentiation, improving combined therapies.

**Keywords**
Histamine • Acute myeloid leukemia • Chemotherapy • Cell differentiation

## 1 Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic progenitor cells arising in the bone marrow that fail to differentiate, to respond to normal regulators of proliferation, and that do not undergo programmed cell death or apoptosis. Leukemic cells that interfere with normal hematopoiesis can escape into the peripheral blood and result in organ infiltration, most threateningly the CNS and lung. This malignant alteration is characterized by a loss of normal hematopoietic function leading to bone marrow failure that is the most common underlying cause of death. The genetic reprogramming of leukemic cells renders them ineffective at generating mature neutrophils, monocytes, red cells, and platelets. Thus, the main sign of bone marrow failure is infection caused by a large range of pathogens including gram-positive and gram-negative bacteria, *Candida* species, and *Aspergillus* species (Anderlini et al. 1996).

It has been estimated about 20,830 new cases of AML and 10,460 deaths only in the United States for 2015 (Siegel et al. 2015). AML is more common in the elderly, with a median age at diagnosis of 67 years, but it represents 15–20% of childhood acute leukemias (Pui et al. 2004). Risks factors for acquiring AML include exposure to ionizing radiation, benzene, and cytotoxic chemotherapy. Almost 15% of patients with AML develop the disorder after the use of chemotherapy for solid cancer treatment.

There are two main systems that have been used to classify AML: The French-American-British (FAB) classification and the World Health Organization (WHO) classification. Depending on the cell type from which leukemia develops and how

**Table 1** WHO classification of AML

| | |
|---|---|
| *AML with certain genetic abnormalities* | AML with a t(8;21) RUNX1-RUNX1R1 |
| | AML with a t(16;16) or inv(16) CBFB-MYH11 |
| | AML with a t(9;11) MLL-AF9 |
| | APL (M3) with a t(15;17) PML-RARA |
| | AML with a t(6;9) DEK-NUP214 |
| | AML with a t(3;3) or inv(3) EVI1-RPN1 |
| | AML (megakaryoblastic) with a t(1;22) RBM15-MKL1 |
| *AML with myelodysplasia-related changes*<br>*AML related to previous chemotherapy or radiation*<br>*AML not otherwise specified*<br>Cases of AML that do not fall into one of The above groups and is similar to the FAB classification | Undifferentiated AML (M0) |
| | AML without maturation (M1) |
| | AML with maturation (M2) |
| | Acute myelomonocytic leukemia (M4) |
| | Acute monocytic leukemia (M5) |
| | Acute erythroid leukemia (M6) |
| | Acute megakaryoblastic leukemia (M7) |
| | Acute basophilic leukemia |
| | Acute panmyelosis with fibrosis |
| *Myeloid sarcoma* | Also known as granulocytic sarcoma or chloroma |
| *Myeloid proliferations related to down syndrome*<br>*Undifferentiated and biphenotypic acute leukemias* | Leukemias that have both lymphocytic and myeloid features |
| | Also called ALL with myeloid markers, AML with |
| | Lymphoid markers, or mixed phenotype acute leukemias |

*t* translocation, *inv* inversion

mature the cells are, FAB system divides AML into subtypes from M0 to M7. On the other hand, WHO classification is newer and defines subsets of AML based on morphologic and cytogenetic characteristics (Table 1).

AML treatment options depend on the subtype as well as on the prognostic features. However, in the last decades, chemotherapy has been the treatment of choice, sometimes followed by allogeneic hematopoietic stem cell transplantation. One of the major complications of chemotherapy is that the current drugs are highly toxic and poorly tolerated, especially by older patients (Estey and Döhner 2006). In general, treatment for AML consists of induction chemotherapy (combination of cytarabine and the anthracycline drugs), less frequently central nervous system prophylaxis (to prevent CNS relapse), and post-remission therapy. Up to 70% of patients will achieve remission with the induction protocol; however, if no further post-remission is given, almost all patients will eventually relapse. Remission rates and overall survival depend on different features among them: age of the patient, cytogenetics (chromosomal aberration), secondary molecular changes within the leukemic clone, previous bone marrow disorders (e.g., myelodysplasia), and comorbid illnesses.

Over the last few decades, the concept of differentiation therapy, whereby immature cells may be stimulated to develop into their mature phenotype, aroused considerable interest. Many efforts are in progress to evaluate new differentiation drugs for the treatment of leukemia in which early hematopoietic progenitors appear to exhibit maturation arrest. Treatment of acute promyelocytic leukemia (APL) with the differentiation agents, vitamin A metabolite all-trans-retinoic acid (ATRA) (Nowak et al. 2009) or arsenic trioxide ($As_2O_3$) (Chou et al. 2005), has been successfully applied. In addition, factors that increase cAMP-mediated signaling, such as cyclic nucleotide phosphodiesterase (PDE) inhibitors, augment the ability of these approved therapies to induce differentiation in APL blast cells (Lerner and Epstein 2006).

## 2 $H_2$ Receptor Signaling and Physiology

The fact that classic antihistamines were not able to block histamine-induced gastric secretion led the researchers to hypothesize the existence of a new histamine receptor subtype (Ash and Schild 1966). Some years later, this hypothesis was confirmed after the development of specific ligands able to block gastric acid secretion (Black et al. 1972), naming this new receptor subtype as $H_2$ receptor.

Numerous studies had found that $H_2$ receptors act as potent stimulators of intracellular cAMP accumulation (Leurs et al. 1995; Hill et al. 1997; Panula et al. 2015). It has been demonstrated that the modulation of cAMP levels occurs via the coupling and activation of Gαs G-protein subunit. This was experimentally demonstrated by [α-$^{32}$P]GTP labeling of Gαs subunits after receptor stimulation in mammalian and insect cell expression systems (Kühn et al. 1996; Leopoldt et al. 1997), by using receptor-G-protein fusion chimeras, [$^{35}$S]GTPγS binding, and steady-state GTP hydrolysis (Kelley et al. 2001; Wenzel-Seifert et al. 2001).

In addition to Gαs coupling to adenylyl cyclase, $H_2$ receptors couple to other signaling systems. It has been shown that $H_2$ receptors couple also to Gαq/11 proteins, resulting in inositol phosphate formation and increases in cytosolic $Ca^{2+}$ concentration in some $H_2$ receptor-expressing cells. Experiments equivalent to those used to demonstrate receptor coupling to Gαs have shown that $H_2$ receptor can also activate Gαq proteins in both mammalian and insect cells (Kühn et al. 1996; Leopoldt et al. 1997). In gastric parietal cells, HL-60 cells, and hepatoma-derived cells transfected with the canine $H_2$ receptor cDNA, $H_2$ receptor stimulation has been shown to increase the intracellular free concentration of calcium ions (Chew 1985, 1986; Malinowska et al. 1988; Mitsuhashi et al. 1989; Chew and Petropoulos 1991; Delvalle et al. 1992; Seifert et al. 1992). Interestingly, $H_2$ receptor coupling to Gαq has been found in rat mammary carcinoma and undifferentiated rat mammary cells and in human breast epithelial cell lines. In these cases, the alternate coupling was correlated with the differentiation cell stage suggesting a relationship between $H_2$ receptor coupling to Gαq and the loss of a regulatory mechanism of cell growth (Davio et al. 1995a, b, 2002).

In addition, in CHO cells transfected with the rat but not human $H_2$ receptor, receptor stimulation produces both an increase in cAMP accumulation and an inhibition of P2u-receptor-mediated arachidonic acid release (Traiffort et al. 1992; Leurs et al. 1994). These observations suggest that these effects might depend on the level of receptor expression or subtle differences between clonal cell lines.

As many other GPCRs, $H_2$ receptor signaling is tightly regulated by receptor desensitization and internalization after agonist stimulation (Smit et al. 1996; Fukushima et al. 1997). Desensitization of the $H_2$ receptor involves both GPCR kinases GRK-2 and GRK-3 but not GRK-5 or GRK-6 (Rodriguez-Pena et al. 2000; Shayo et al. 2001). Remarkably, the regulation of the $H_2$ receptor by GRK-2 relies on a dual mechanism, while the kinase activity is implicated in receptor internalization and recycling, the RGS (regulator of G-protein signaling) homology domain of GRK-2 is responsible for $H_2$ receptor desensitization (Fernandez et al. 2011).

Interestingly, GRK-2-mediated desensitization has proved to be involved in the lack of hematopoietic cell maturation promoted by $H_2$ receptor stimulation. When GRK-2 is downregulated, $H_2$ receptor-mediated cAMP response is higher and more sustained, allowing cells to differentiate after treatment with $H_2$ agonists (Fernández et al. 2002). This fact results therapeutically relevant and will be extensively discussed later. Concerning receptor internalization, a role of dynamin, β-arrestin, and clathrin has also been reported (Fernandez et al. 2008), and the GTPase dynamin has been identified as a binding partner for the $H_2$ receptor, both in vitro and in vivo (Xu et al. 2008).

Regulation of gastric acid secretion represents the paradigmatic function of histamine that is mediated by the activation of $H_2$ receptors. However, along the years, several other functions of histamine were assigned to its action over $H_2$ receptor. In addition to the stomach, the $H_2$ receptor is expressed in the brain, smooth and cardiac muscle cells, chondrocytes, endothelial and epithelial cells, neutrophils, eosinophils, monocytes, macrophages, dendritic cells, and T and B cells (Jutel et al. 2009).

Histamine has been typically considered an effector molecule for chronic and immediate hypersensitivity (Pearce 1991). However, growing evidence suggest that it is a potent modulator of the immune system. At low physiological concentrations, histamine can act as an immunostimulant exerting its action mainly through $H_1$ receptors. On the other hand, at higher concentrations, histamine released by basophils, mastocytes, or tumor cells acts as immunosuppressor through $H_2$ receptors, activating suppressor T cells and inhibiting T helper cytokine production (Jutel et al. 2006). Histamine also inhibits the production of reactive oxygen species (ROS) in isolated monocytes, neutrophils, and leukemic cells recovered from patients with myelomonocytic and monocytic forms of AML (FAB classes M4 and M5) (Hellstrand et al. 1994; Ching et al. 1995; Reher et al. 2012; Aurelius et al. 2012; Werner et al. 2014). This effect on ROS production has a great impact on clinical use of $H_2$ ligands to treat hematopoietic-related malignancies in general and AML in particular and will be further discussed. Remarkably, it has been reported that the effect on the oxidative burst of granulocytes and monocytes is

not mediated by cAMP accumulation, and it has been provided substantial evidence for ligand-specific conformations of the $H_2$ receptor, suggesting that $H_2$ receptor-biased signaling might be an important concept to consider for clinical treatment design (Reher et al. 2012; Werner et al. 2014).

## 3 Histamine Dihydrochloride and $H_2$ Agonists for the Treatment of Acute Myeloid Leukemia

Signs and symptoms of AML are caused by the lack of normal blood cells and their replacement with leukemic cells. Although the leukemic cells themselves are derived from white blood cell precursors, they have no infection-fighting capacity, and therefore AML makes the patient susceptible to infections (Anderlini et al. 1996).

The pathophysiology of AML permits to envisage at least two treatment strategies, the most obvious and conventional involves chemotherapy, aiming to kill malignant cells. However, due to the high toxicity and lack of specificity of most chemotherapeutic agents, an alternative therapy has been suggested based on the possibility of differentiate abnormal undifferentiated malignant cells to their differentiated counterparts. This strategy allows acquiring the lineage specificity and functional characteristics of mature cells. This approach is termed "differentiation therapy" and is based on the hypothesis that many neoplastic cell types exhibit reversible defects in the course of differentiation, which, upon appropriate treatment, result in tumor reprogramming with a concomitant loss of proliferative capacity and induction of terminal differentiation or apoptosis (Nowak et al. 2009).

As discussed before, histamine receptors have a role in immune cell life cycle and differentiation (Jutel et al. 2006, 2009), making them suitable targets for the treatment of AML. With varied results, both strategies are in different steps of development. They are depicted in Fig. 1 and will be discussed below.

### 3.1 Histamine Dihydrochloride as Chemotherapy Complement

AML first-line treatment is primarily chemotherapy that is divided into two phases: induction and post-remission (or consolidation) therapy. The goal of the first phase is to reach a complete remission, meaning that no disease can be detected with available diagnostic methods (i.e., to reduce the number of leukemic cells to an undetectable level). The length of remission depends on the prognostic features of the original leukemia, and although chemotherapy induces remission in up to 80% of patients with de novo AML, in general, all remissions will fail without additional consolidation therapy (Grimwade et al. 1998, 2001; Farag et al. 2006). Therefore, more therapy is necessary to eliminate non-detectable malignant cells and prevent relapse, that is, to achieve a cure.

Natural killer (NK) cells are an important component of the innate immune system, providing first-line defense against virus-infected cells and tumors. NK

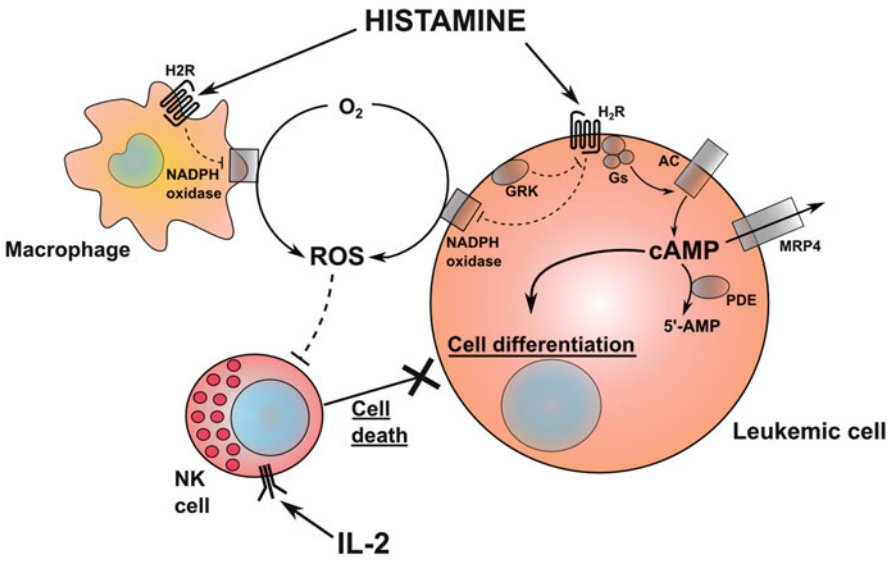


**Fig. 1** Histamine actions on leukemic cell fate. Histamine or $H_2$ agonists increasing intracellular cAMP levels are able to induce leukemic cell differentiation. To achieve the effect, GRK-2-mediated $H_2$ receptor desensitization, PDE-mediated cAMP degradation, and/or MRP4-mediated second messenger efflux should be inhibited. Histamine is also able to inhibit macrophage and leukemic cells ROS production allowing IL-2 activation of NK cells or T cytotoxic cells with the consequent leukemic cell death. *Arrows* indicate activation, while *dotted lines* indicate inhibition

cells are cytotoxic to AML blasts as demonstrated by the graft-versus-leukemia effect in patients with leukemia after bone marrow transplantation (Lotzová et al. 1987; Barrett 2008), and higher NK-mediated cytotoxicity has been reported to result in higher leukemia-free survival (Lowdell et al. 2002). Moreover, there are studies suggesting that NK cells may be compromised in AML (Costello et al. 2002; Fauriat et al. 2007). These observations support the role of these cells in AML progression and are suggestive about their prognostic value helping to the accurate prediction of disease outcome.

In this regard, interleukin-2 (IL-2) is a key cytokine in the activation of T and NK cells (Waldmann 2006), and it is indicated for the treatment of metastatic renal cell carcinoma and metastatic melanoma (Proleukin®, Novartis Pharmaceuticals Corporation). However, any significant advantage of the use of IL-2 over no treatment could not be demonstrated in large, randomized trials in patients with AML (Blaise et al. 2000; Baer et al. 2008; Pautas et al. 2010; Kolitz et al. 2014). This lack of in vivo efficacy in patients can be attributed to "tumor-induced immunosuppression" of NK cells (Hellstrand 2002). Tumor-associated macrophages and leukemic cells recovered from patients with myelomonocytic and monocytic forms of AML (FAB classes M4 and M5) convert oxygen into ROS, and these free radicals create a reduced environment that impedes the activation of

NK cells, including that by IL-2 (Murdoch et al. 2004; Romero et al. 2009; Aurelius et al. 2012).

The mechanism of action of histamine through $H_2$ receptors in AML consists in the inhibition of the activity of NADPH oxidase and the consequent production of ROS by tumor-associated monocytes and by leukemic cells themselves, conferring protection from tumor-induced immunosuppression (Hellstrand 2002; Romero et al. 2009; Martner et al. 2010). Therefore, the addition of histamine dihydrochloride to IL-2 enables the activation of T cells and NK cells by IL-2 (Hellstrand et al. 1994). In vitro studies have shown that this effect of histamine is mimicked by the $H_2$-specific agonist dimaprit and blocked by the addition of the $H_2$ antagonists ranitidine or cimetidine (Hellstrand and Hermodsson 1986; Brune et al. 1996). It is worth noting that IL-2 plays also a crucial role in Treg cells boosting immune regulation. IL-2-dependent activation of NK and T cytotoxic cells depends on the application of high doses of IL-2, while infusion of relatively low doses of IL-2 seems to selectively produce Treg cells boosting immune suppressive mechanisms (Malek and Bayer 2004). This balance between immune activation and suppression by IL-2 should be considered when therapeutic schemes are to be developed. In addition, it has been described that histamine acting on $H_2$ receptors, and independently of cAMP modulation, is able to decrease the high constitutive activity of Akt2 in U937 cells (Werner et al. 2016). These observations are very significant considering that phosphorylation of this kinase is crucial for the regulation of numerous downstream targets involved in cell growth, proliferation, survival, differentiation, and metabolism (Martelli et al. 2009; Vivanco and Sawyers 2002). Since Akt activation promotes AML progression (Martelli et al. 2006; Vivanco and Sawyers 2002) and it is associated with a shorter overall survival (Gallay et al. 2009; Min et al. 2003), it cannot be discarded the inhibition of Akt2 activation as a complementary mechanism by which histamine achieves its effects as a potential clinical treatment for post-remission therapy.

As a widely distributed local mediator and neurotransmitter, histamine acts on a multitude of cell types in addition to cells of the immune system and blood cells, including smooth muscle cells, neurons, and endocrine and exocrine cells, having many systemic effects, mediated mainly by $H_1$ and $H_2$ receptors such as anaphylaxis, vasodilation, gastric acid secretion, and neurotransmission (Panula et al. 2015). Consequently, the use of subcutaneous histamine dihydrochloride may result in vasodilation and hypotension and other related adverse events.

Information about the tolerability of histamine dihydrochloride with or without concomitant IL-2 was obtained from the phase III trial in patients with AML in complete remission (Brune et al. 2006), reviews (Mekhail et al. 2000), and the EU summary of product characteristics (http://www.ema.europa.eu). Since histamine is a potent vasoactive agent, the use of histamine dihydrochloride has been frequently ($>30\%$) associated with flushing, headache, fatigue, and pyrexia (Hellstrand 2002). Other less frequent vasodilatation-related adverse events include hypotension and tachycardia (Martner et al. 2010). Because histamine dihydrochloride and IL-2 are administered by subcutaneous injection, injection-site adverse events such as injection-site granuloma and erythema may occur, and this type of reaction is the

most common cause of dose reduction or treatment interruption (Brune et al. 2006). Anyway, in the phase III trial, combined histamine dihydrochloride and IL-2 therapy had an acceptable tolerability profile.

At his point, histamine tolerability needs to be established in a wider AML population, not restricted to stringent clinical trial inclusion criteria, and over the longer term. Moreover, the use of specific $H_2$ agonists instead of histamine could constitute a genuine strategy to avoid undesired effects produced by the activation of other histamine receptor subtypes. In conclusion, histamine dihydrochloride and IL-2 as post-consolidation immunotherapy significantly prolonged leukemia-free survival compared with no treatment having an acceptable tolerability profile and seems to be a useful therapy option for adult patients with AML in remission.

## 3.2 $H_2$ Histamine Ligands as Leukemic Differentiation Agents

As mentioned before, the aim of differentiation therapy is to reprogram neoplastic cells with a treatment that suppresses the exacerbated proliferative capacity of tumor cells and induces terminal differentiation or apoptosis. Differentiation induction as a therapeutic strategy has the greatest impact on hematopoietic malignancies, most notably on leukemia.

Treatment of acute promyelocytic leukemia with differentiation agents such as vitamin A metabolite all-trans-retinoic acid (ATRA) (Nowak et al. 2009) or arsenic trioxide (As2O3) (Chou et al. 2005) has been successfully applied. In addition, factors that increase cAMP-mediated signaling, such as cyclic nucleotide phosphodiesterase (PDE)-4 inhibitors, augment the ability of these approved therapies to induce differentiation in acute promyelocytic leukemia blast cells (Lerner and Epstein 2006). Efforts to identify others and potentially more effective differentiation inducers for the treatment of leukemia have remained a focus of major interest.

Cyclic AMP was the first second messenger reported, and since then numerous studies have shown its participation in many physiological and/or pathophysiological processes including cell cycle regulation. The signaling pathway mediated by this cyclic nucleotide has emerged as a key regulator of blood cell proliferation, differentiation, and apoptosis in malignant cell populations (Kobsar et al. 2008).

Cyclic AMP-elevating agents, including histamine $H_2$ agonists, are able to induce granulocyte differentiation in the human promyelocytic cell line HL-60 (Chaplinski and Niedel 1982; Nonaka et al. 1992). In M1 mouse myeloid leukemia cells as well as in the human promonocytic leukemia U937 cell line, dibutyryl cAMP (db-cAMP) but not $H_2$ agonists induces cell maturation (Honma et al. 1978; Shayo et al. 1997). In this regard, it was demonstrated the important role of the kinetic of the cAMP signaling in U937 cell differentiation (Lemos Legnazzi et al. 2000; Shayo et al. 2004). Interestingly, cAMP can also potentiate granulocytic differentiation of ATRA- or arsenic trioxide-induced maturation of human APL cells (Zhu et al. 2002; Guillemin et al. 2002; Nguyen et al. 2013).

Despite diverse extracellular signals activate GPCRs leading to an increase in cAMP, signal specificity results from accurate adjustments at different levels of the

cAMP-dependent pathway. Although cAMP is increased following $H_2$ receptor stimulation, in some leukemic cells differentiation fails to occur due to rapid receptor desensitization. Recently, cAMP efflux across MRP transporters was described in several systems as a regulator of intracellular cAMP levels modulating biological responses (Osycka-Salut et al. 2014; Copsel et al. 2014; Ventimiglia et al. 2015; Decouture et al. 2015). Both desensitization and extrusion processes will be discussed below.

However, it is important to consider recent reports indicating that cAMP can promote AML progression and protect myeloid leukemia cells against anthracycline- and arsenic trioxide-induced apoptosis (Gausdal et al. 2013; Safa et al. 2014). This suggests that the beneficial pro-differentiating and non-beneficial pro-survival effects of cAMP should be weighed against each other.

### 3.2.1 $H_2$ Receptor Desensitization Process as Pharmacological Target

Cyclic AMP is generated following the interaction of ligands with a receptor coupled to a transducer G protein. The occupied receptor promotes the exchange of GTP in the transducer, thus generating an activated subunit, which in turn activates the effector adenylyl cyclase (Marinissen and Gutkind 2001). The activation of this membrane signal transduction machinery is transient because several mechanisms are activated to terminate the stimulation and to return the cell to a resting state. These include the phosphorylation of the receptor by different kinases and the recruitment of β-arrestins, or inactivation of Gs via hydrolysis of GTP at a rate controlled by the regulators of G-protein signaling (RGS) protein (Freedman and Lefkowitz 1996). Activation of phosphodiesterases (PDEs) that are downstream of receptor/G-protein/effector coupling is an additional regulatory mechanism that induces the termination of the stimulus distal to the generation of cAMP (Conti et al. 1991).

Knowing that intracellular cAMP levels are important for leukemic differentiation, it is reasonable to assume that by targeting the mechanisms that regulate its intracellular levels, it would be possible to influence the ability of leukemic cells to be differentiated. In this sense, a proof of concept was to stably overexpress $H_2$ receptor to induce leukemic cell differentiation. In U937 cells, $H_2$ receptor overexpression triggered several mechanisms (namely, PDE activity induction and GRK-2 overexpression) tending to restore cAMP basal levels comparable to those of the naïve cells. The results obtained in time-course, dose-response, and desensitization experiments suggest that the mechanisms elicited as a consequence of receptor overexpression are able to manage cAMP basal levels but are not able to handle cAMP levels in stimulated conditions.

In spite of the onset of these regulatory mechanisms, the higher and sustained increase of cAMP levels caused by $H_2$ agonists in $H_2$ receptor overexpressing U937 cells induces differentiation and hampers the proliferation of the overexpression clone (Monczor et al. 2006). These findings provide new insights into the relevant role of receptor stoichiometry in the effector regulation on cell behavior and further suggest that this regulation may be externally manipulated to achieve beneficial therapeutic effects in the future.

There are seven members of the GRK family: GRK-1 through GRK-7. On the basis of sequence homology, these can be classified into three groups: GRK-1 (also known as rhodopsin kinase), GRK-2 and GRK-3 (also called β-adrenergic receptor kinases 1 and 2), and finally GRK-4, GRK-5, GRK-6, and GRK-7. The mechanisms by which GRK activity is regulated can be divided into three categories: subcellular localization, alterations in intrinsic kinase activity, and alterations in GRK expression levels. Cytosolic GRK-2 and GRK-3 are translocated to the membrane after receptor activation, in a process facilitated by the interaction with released Gβγ dimers (Palczewski 1997; Penn et al. 2000). Although GRK-2, GRK-3, GRK-5, GRK-6, and GRK-7 subtypes are ubiquitous, GRK-2 is particularly abundant in peripheral blood leukocytes and in myeloid and lymphoid cell lines (Chuang et al. 1992). GRK expression is tightly regulated and can be altered by different extracellular factors (Penela et al. 2003). It has also been demonstrated that their expression can be modified as a compensatory mechanism when the expression of one member is modified (Fernandez et al. 2007).

In U-937 leukemic cell line, the decrease in GRK-2 expression correlates with an increase of cAMP levels in response to different doses of $H_2$ agonist, in time-course cAMP accumulation experiments, and in desensitization assays. Hence, the reduction in GRK-2 expression determined a higher and prolonged cAMP response mediated by $H_2$ ligands allowing leukemic cell differentiation upon $H_2$ agonist treatment. These results establish an important correlation between duration and intensity of a signal and cellular response, showing that as a consequence of modulating the desensitization process, cells are able to switch from proliferation to differentiation pathway (Fernández et al. 2002). Overall, it can be concluded that GRK-2 plays a fundamental role modulating $H_2$ receptor signaling and that this kinase is to be considered a pharmacological target that, when intervened, is able to determine cell differentiation.

Structurally, GRK-2 protein contains an N-terminal RGS-homology domain (RH), a catalytic central domain (Kin), and a C-terminal region responsible for membrane localization (Penela et al. 2003). More recent experiments showed that the RGS domain and not kinase activity is necessary for $H_2$ receptor desensitization (Fernandez et al. 2011). This dual role of GRK-2 involving both functional domains (Kin and RH) is depicted in Fig. 2.

### 3.2.2 Cyclic AMP Efflux Mediated by MRP4 as a Target in Acute Myeloid Leukemia

Multidrug resistance protein 4 (MRP4) belongs to the C-branch of the superfamily of ATP-binding cassette transporters (ABC, ABCC4). These transporters are capable of actively pumping a wide range of endogenous and xenobiotic substrates out of the cells (Deeley et al. 2006). In particular, MRP4 has the ability to transport a broad variety of drugs including antivirals (adefovir, ganciclovir, tenofovir), antibiotics (cephalosporins), cardiovascular (thiazides, furosemide), and chemotherapeutic (methotrexate, 6-mercaptopurine, 6-thioguanine, topotecan) (Russel et al. 2008). However, the pathophysiological actions of these proteins are quite diverse, and transport of cytotoxic xenobiotics as a defense mechanism appears not

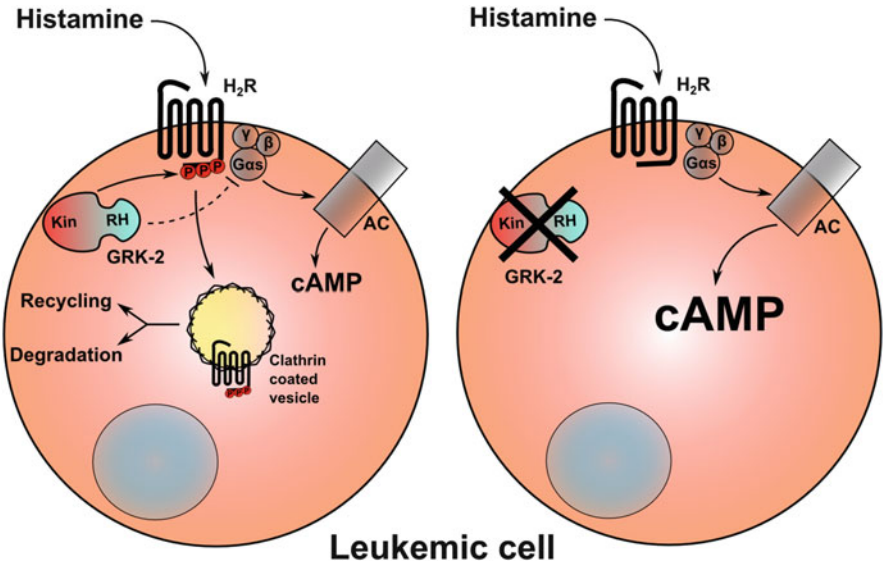


**Fig. 2** Dual regulation of H2 receptor signaling by GRK-2. In the *left panel*, GRK-2 is able to regulate H2 receptor signaling through the activity of two functional domains. While the domain with kinase activity (Kin) phosphorylates the receptor in its C-term tail (*circled* Ps) inducing receptor internalization and recycling, the RGS-homology domain (RH) directly interacts with the G-protein inhibiting its activity. As mentioned in the main text, both processes modulate cAMP receptor signaling and make of GRK-2 a suitable target for inducing leukemic cell differentiation. In the right panel, as a consequence of GRK-2 downregulation or inhibition, H2 receptor signaling is increased and sustained over the time

to be the only important evolutionarily conserved function. Moreover, while several members of the ABC family are established as drug transporters, others also mediate transport of endogenous molecules. Indeed, MRP4 is capable of transporting a wide range of endogenous and signaling molecules including folates, bile acids, conjugated steroids, purine analogs, eicosanoids (prostaglandin E2, thromboxane TXB2, and leukotriene B4), ADP, and cyclic and nucleotides (cAMP and cGMP) (Russel et al. 2008). Remarkably, MRP4 is the major cAMP efflux transporter, and as already said, this cyclic nucleotide is involved in the regulation of cellular proliferation, differentiation, and apoptosis (Karin 1994). Recently, MRP4 mRNA and protein expression were found to be regulated by cAMP in Hela cells, vascular smooth muscle cells, megaloblastic leukemia M70e cells, and pancreatic adenocarcinoma cell lines (Bröderdorf et al. 2014; Carozzo et al. 2015). MRP4 expression is regulated through a mechanism where the balance between intracellular and extracellular cAMP plays a key role in the feedback regulation of the transporter expression. Persistent cAMP intracellular levels induce MRP4 promoter through the exchange proteins directly activated by cAMP (EPAC)/Rap1 pathway, whereas extracellular cAMP inhibits it through ERK phosphorylation (Carozzo et al. 2015).

Aside from physiological expression in blood cells, MRP4 has also been found in human leukemia cell lines (Oevermann et al. 2009; Copsel et al. 2011; Takeuchi et al. 2012). As in vitro it was clearly demonstrated that MRP4 confers resistance to nucleoside analog drugs and promotes the efflux of cyclic nucleotides, it has the potential to affect leukemia development and treatment. Therefore, in the last decade, the potential clinical relevance of this transporter has been specially examined in patients with AML. A clinical study for adult AML revealed the expression of MRP4 in blast cells with significant variability. Higher protein levels of this transporter were detected in the less differentiated FAB subtypes M0 and M1; however, its expression has no influence on treatment outcome using cytarabine. Furthermore, MRP4 expression did not correlate with remission rate and overall and relapse-free survival (Guo et al. 2009). On the contrary, a phase II clinical study in adult patients with AML in first relapse treated with gemcitabine and mitoxantrona revealed that higher expression of MRP4 and solute carrier family 29 member A2 correlated with not achieving complete remission (Advani et al. 2010).

When 53 children with de novo AML were evaluated, MRP4 mRNA expression was found in all patients. Nevertheless, as in adult AML, MRP4 in childhood AML was not associated with the failure to achieve remission (Steinbach et al. 2003). Recently, frequent copy number alterations of MRP4 were observed in de novo AML, and variable expression of this transporter was detected among AML subtypes from 155 pediatric patients. Although some authors found the highest levels of MRP4 in the less differentiated AML subtypes, in this study, MRP4 expression was found to be higher in the M7 AML subtype (Lian et al. 2013).

As MRP4 is the major cAMP efflux transporter, current evidences suggest that MRP4 is implicated not only in chemotherapy resistance but also in cancer biology. Indeed, the mere genetic silencing or pharmacologic inhibition of MRP4 reduced tumor growth in a xenograft AML model. Furthermore, MRP4 knockdown induced cell cycle arrest and apoptosis in vivo (Copsel et al. 2014). As it was mentioned above, the finding that MRP4 overexpression confers nucleoside analog drugs resistance has strong implications for leukemia chemotherapy (Adachi et al. 2002).

In particular, MRP4 expression was detected in KG-1, HL-60, U937, KG-1a, and AML cell lines, and its expression decreases during leukocyte differentiation promoting cAMP accumulation in differentiated cells (Oevermann et al. 2009; Takeuchi et al. 2012). In accordance, it was demonstrated that besides playing a role in drug-resistant leukemia cell lines, MRP4 regulates leukemia cell proliferation and differentiation through the endogenous MRP4 substrate, cAMP (Copsel et al. 2011). The signaling pathway mediated by this cyclic nucleotide has emerged as a key regulator of blood cell proliferation, differentiation, and apoptosis in malignant cell populations (Kobsar et al. 2008). Thus, $H_2$ agonist when combined with MRP4 and PDE4 inhibitors induces cell cycle arrest and maturation in U937 cells. By using two well-characterized MRP inhibitors such as probenecid and MK571 in intact cells and membrane vesicles, it has been shown that MRP inhibition further enhanced $H_2$ receptor-induced intracellular cAMP concentration, allowing cell growth inhibition and differentiation. MRP pharmacological

inhibition or knockdown modified the intracellular content of cAMP concomitantly with an accentuated decrease in the proliferative rate of U937 cells. This inhibition was even more pronounced when MRP inhibitors were combined with cAMP-stimulating agents, such as $H_2$ receptor agonists (Copsel et al. 2011; Werner et al. 2015).

Altogether these findings indicate that agents that modulate or mimic cAMP levels should be considered as a new alternative strategy for AML treatment, either alone or in combination with chemotherapeutic drugs.

## 4 Final Considerations

Histamine, as a wide distributed local mediator and neurotransmitter, mediates many cell functions and its receptors are potential targets for the treatment of several diseases. Hematopoietic cells express histamine receptors, and their modulation has the potential to ameliorate their pathologies. Among years, histamine ligands prove to be of clinical utility and are among the top marketed drugs around the world. This did not prevent the search and finding of novel therapeutic uses, providing promising results concerning cancer treatment, specifically involving AML. Up to now, two main strategies have been pursued: the complementation of chemotherapeutics to allow immune rejection of cancer cells in a graft-versus-host type of reaction and the induction of differentiation of malignant cells to eliminate abnormal cell proliferation and to induce terminal differentiation recovering the functionality of the original tissue. Thus, the treatment with histamine or $H_2$ agonists in combination with IL-2 or GRK2, PDE4, or MRP4 inhibitors represents a therapeutic scheme with great potentiality. The results obtained in preclinical and clinical studies grant further research to achieve optimized treatments with fewer side effects.

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