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Butirato Sódico Potencia os Efeitos Citotóxicos de Antineoplásicos sobre Células T Linfoblásticas Humanas

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Sumário

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Dedico esta conquista...

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Resumo

Butirato sódico (NaB), um potente inibidor da desacetilação de histonas, inibe o crescimento celular e induz apoptose em células neoplásicas. Investigou-se, in vitro, os efeitos na proliferação celular do tratamento combinado de NaB e fármacos comumente empregados no tratamento de leucemias. A linhagem de células T linfoblásticas, Jurkat, foi cultivada em meio RPMI suplementado com 10% de FBS. As células foram tratadas com bleomicina, citarabina, doxorubicina, etoposideo, metotrexato, vincristina e NaB. A proliferação e viabilidade celular foram avaliadas 48h após os tratamentos. Nossos resultados mostraram que NaB aumenta os efeitos citotóxicos de citarabina e etoposideo, mas não de bleomicina, doxorubicina, metotrexato ou vincristina. Estes dados sugerem que NaB é um promissor agente terapêutico adjuvante para o tratamento de leucemias linfoblásticas, e proporcionam uma base para futuras investigações neste campo.

Palavras-chave: leucemia linfoblástica aguda, butirato sódico, tratamento adjuvante, quimioterapia.

Introdução

Leucemia linfoblástica aguda (LLA), uma doença maligna de células linfóides progenitoras, afeta tanto crianças quanto adultos, com um pico de prevalência em indivíduos com idades entre 2 e 5 anos. Os fármacos freqüentemente utilizados para tratar leucemias foram inicialmente desenvolvidos e testados entre 1950 e 1970. Embora o constante progresso no desenvolvimento de terapias anti-câncer tenha proporcionado uma cura de aproximadamente 80% em crianças, os fármacos são freqüentemente utilizados em doses elevadas, provocando diversos efeitos colaterais e toxicidade nestes pacientes. Assim, esforços no sentido de identificar novos tratamentos são justificados (Pui et al., 2008).

O balanço entre acetilação e desacetilação de histonas é um importante fator na regulação da expressão gênica eucariótica. Inibidores da desacetilação de histonas (HDIs), como o butirato sódico (NaB), estão em evidência como uma potencial nova classe de agentes antineoplásicos. Isto se deve ao fato destas substâncias promoverem ou auxiliarem uma variedade de diferentes mecanismos anti-câncer, como apoptose, parada do ciclo celular e diferenciação celular. Ensaios clínicos recentes têm demonstrado que a hiperacetilação de histonas pode ser realizada com segurança em humanos, e que o tratamento com estes agentes é plausível. Portanto, a utilização de HDIs em associação com fármacos antineoplásicos clássicos ou em combinação com agentes demetilantes de DNA representa uma promissora alternativa de tratamento (Kouraklis & Theocharis, 2006).

Em leucemia linfoblástica aguda, ainda permaneciam desconhecidos os efeitos que fármacos antineoplásicos clássicos e HDIs poderiam provocar na proliferação celular. Além disso, a utilização de HDIs em associação com estes fármacos clássicos, que representa uma promissora alternativa de tratamento, também não havia sido devidamente estudada neste tipo de câncer. O presente estudo propôs-se a avaliar *in vitro* o efeito anti-proliferativo obtido através do tratamento combinado de NaB com fármacos anti-câncer clássicos, comumente utilizados para tratar leucemias, na linhagem celular linfoblástica humana, Jurkat.

Revisão da Literatura

Leucemias

Leucemia é um câncer, doença maligna, da medula óssea e do sangue que afeta tanto crianças quanto adultos, sendo comumente dividida em quatro categorias: mielóide aguda (LMA) ou mielóide crônica (LMC), envolvendo a linhagem mielóide da medula óssea (leucócitos, eritrócitos e megacariócitos) e linfoblástica aguda (LLA) ou linfoblástica crônica (LLC), envolvendo células da linhagem linfóide.

As leucemias, que correspondem a 30% dos tumores oncopediátricos, constituem as neoplasias mais freqüentes em indivíduos com menos de 15 anos (Belson et al., 2007). A LLA, uma doença maligna de células linfóides progenitoras, com um pico de prevalência em indivíduos com idades entre 2 e 5 anos, é o tipo mais comum de câncer infantil, representando 75-80% de todos os casos de leucemias, e cerca de um terço de todas as neoplasias malignas da criança (Pui, 2000; Redaelli et al., 2005; Pui et al., 2008).

Os tratamentos padrão para todos os tipos de leucemias geralmente envolvem quimioterapia e/ou transplante de medula óssea e/ou radioterapia. Embora os esquemas terapêuticos possam mudar de centro para centro, os protocolos atuais são geralmente constituídos por quatro componentes: indução, intensificação-consolidação, manutenção e prevenção da leucemia no sistema nervoso central (SNC). Diversos fármacos citotóxicos são empregados no tratamento, sendo que os mais utilizados são metotrexato, vincristina, doxorubicina, daunorubicina, L-asparaginase, 6-mercaptopurina, etoposideo,

citarabina e ciclofosfamida (Tabela 1), sendo que o tratamento geralmente envolve a combinação de dois ou mais destes antineoplásicos. (Radaelli et al., 2005).

Tabela 1. Fármacos citotóxicos clássicos freqüentemente utilizados no tratamento de leucemias e seus mecanismos de ação. A classe do fármaco está em itálico, seguida pelo nome do fármaco em negrito.

Mecanismo de Ação			**Fármacos Clássicos para Tratar Leucemia**
Inibidor Mitotico (Fase M)	Bloqueadores da formação de microtubulos		*Alcalóides* da *vinca*: **Vincristina**
inibidor de replicação de DNA	Precursores de DNA / antimetabólitos (Fase S)	Ácido fólico	*Inibidor de diidrofolato redutase* : **Metotrexato**
		Purina	*Tiopurina*: **Mercaptopurina**
			Halogenados/ inibidores de ribonucleotideo redutase : **Cladribina, Clofarabina, Fludarabina**
			Tiopurina: **Tioguanina**
		Pirimidina	*Inibidor de DNA polimerase* **Citarabina**
		Desoxiribonucleotideo	*Inibidor de Ribonucleotideo redutase* : **Hidroxiureia**
	Inibidores de Topoisomerase (Fase S)	II	*Podophyllum*: **Etoposideo, Teniposideo**
		II+Intercalação	*Antraciclinas*: **Daunorubicina, Doxorubicina, Idarubicina**
			Antracenedionas: **Mitoxantrona**
	Ligação cruzada no DNA “Crosslinking”	Alquilantes	*Mustardas nitrogenadas* : **Mecloretamina, Ciclofosfamida, Clorambucila.**
			Alquil sulfonatos: **Busulfano**
		Relacionados aos Alquilantes	*Platinas*: **Carboplatina, Cisplatina**
Outros	Inibidores de enzimas		*Prl*: **Bortezomib**
	Não agrupados		*Retinoides:* **Tretinoina**
			Redutor de asparagina : **Asparaginase**
			Vorinostat

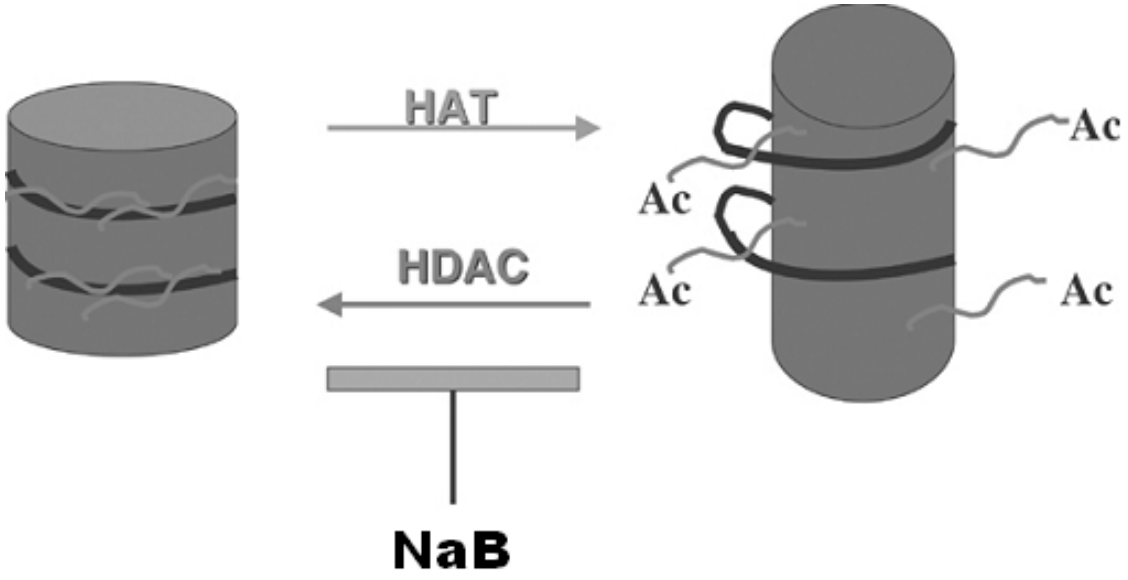
O constante progresso no desenvolvimento do tratamento tem proporcionado índices cada vez melhores de cura, alcançando aproximadamente 80% em crianças com LLA. Entretanto, mesmo neste caso onde se verificam os maiores índices, ainda há um número considerável de recidiva de doença (Den Bôer et al., 1999; Pui, 2000) e, pacientes de alto risco possuem uma expectativa de sucesso terapêutico reduzida, correspondendo a menos de 50% mesmo quando utilizada uma terapia Intensiva (Zago et al, 2001). Além disso, os fármacos freqüentemente empregados no tratamento são utilizados em doses elevadas, provocando diversos efeitos colaterais e toxicidade nestes pacientes. Assim, esforços no sentido de identificar novos quimioterápicos ou agentes adjuvantes para o tratamento de todos os tipos de leucemias ainda são justificados (Pui et al., 2008).

Histonas Desacetilases (HDACs) e Inibidores de Histonas Desacetilases (HDIs)

A organização da cromatina é crucial para a regulação da expressão gênica. Em particular, propriedades e posicionamento do nucleosomo influenciam na transcrição em resposta a sinais extra ou intracelulares. A acetilação e desacetilação de histonas desempenham importante papel na regulação transcricional de células eucarióticas (Zwiebel, 2000), e são catalisadas por famílias especificas de enzimas, as histonas acetil transferases (HATs) e desacetilases (HDACs) respectivamente (Kouraklis & Theocharis, 2002). HDACs são uma familia de enzimas (presentes em bactérias, fungos, plantas e animais) que removem o resíduo acetil dos aminoácidos lisina

presentes na extensão N-terminal do núcleo das histonas. Conseqüentemente aumentam a carga positiva na porção N-terminal do núcleo das histonas, o que reforça a interação com a carga negativa do DNA e bloqueia o acesso da maquinaria transcricional ao DNA. A acetilação neutraliza as cargas das histonas, gerando uma conformação do DNA mais aberta (Figura 1) (Paris et al., 2008).

Figura 1. Acetilação e desacetilação de histonas catalisadas pelas enzimas histonas acetil transferases (HAT) e desacetilases (HDAC) respectivamente. A acetilação neutraliza as cargas das histonas, gerando uma conformação do DNA mais aberta. Inibidores da desacetilação de histonas, como o butirato sódico (NaB), impedem a desacetilação catalisadas pelas HDACs, promovendo a hiperacetilação de histonas.



O balanço entre acetilação e desacetilação de histonas é um importante fator na regulação da expressão gênica e está, então, ligado ao controle do destino celular. Perturbação da atividade de HAT ou HDAC pode estar associada com o desenvolvimento de câncer (Timmerman et al., 2001), e nestes casos os processos moleculares envolvidos com a ativação ou repressão da transcrição são possíveis alvos para terapia anticâncer. HDACs

foram avaliados pela primeira vez em câncer em leucemia promielocítica aguda (Warrell et al., 1998). Desde então, o silenciamento ou inibição de HDAC tem mostrado ter um impacto no ciclo celular, crescimento celular, descondensação da cromatina, diferenciação celular, apoptose e angiogênese em diversos tipos de células de câncer (Paris et al., 2008).

Uma vez que a inibição da atividade de HDAC reverte o silenciamento epigenético freqüentemente observado em câncer, diversos inibidores de HDAC (HDIs) têm sido desenvolvidos para fins terapêuticos (Tabela 2). Estes incluem ácidos graxos de cadeia curta (como ácido valpróico [VPA] e butirato sódico [NaB]), ácidos hidroxâmicos (como Vorinostat [SAHA], Tricostatin A [TSA], Panobinostat [LBH-589], Belinostat [PXD101]), benzamidas (MS-275), e tetrapeptideos cíclicos (como trapoxin, apicidin e depsipeptideo), bem como uma variedade de outros compostos químicos (Bouchain et al., 2003; Jeong et al., 2003; Van Ommeslaeghe et al., 2003; Piekarz & Bates, 2004; Kouraklis & Theocharis, 2006; Carew et al., 2008).

Inibidores de HDACs possuem graus variados de especificidade, embora o mecanismo molecular anticâncer de cada agente específico não esteja completamente claro. A criação de inibidores com maior especificidade permitirá que a função de cada agente seja melhor elucidada, além de melhorar a eficácia e reduzir a toxicidade (Carew et al., 2008). A pesquisa atual na terapia do câncer está focada no desenho de fármacos que agem especificamente contra alterações moleculares encontradas apenas nas células transformadas. O objetivo é associar um tipo específico de tumor com

um determinado perfil de expressão gênica, definindo assim a alteração responsável por cada câncer (Paris et al., 2008).

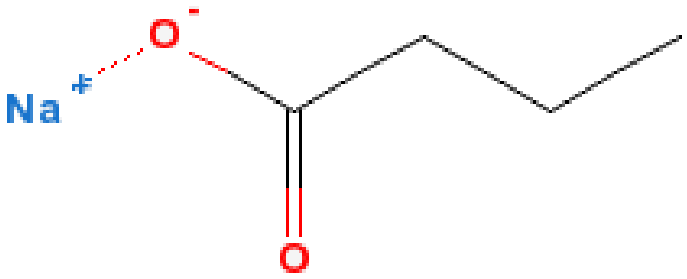
Tabela 2. Principais inibidores de histona desacetilases (HDIs) avaliados como adjuvantes no tratamento de leucemia.

Classe	Composto	Nome Químico (IUPAC)
Ácidos graxos de cadeia curta	Acido valpróico	2-propylpentanoic acid
	Butirato sódico (NaB)	Butanoate
Derivados do ácido hidroxâmico	Vorinostat (SAHA)	N'-hydroxy-N-phenyloctanediamide
	Tricostatin A (TSA)	(2E,4E)-7-(4-dimethylaminophenyl)-N-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide
	Panobinostat (LBH-589)	(E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide
	Belinostat (PXD101)	(E)-N-hydroxy-3-[3 (phenylsulfamoyl) phenyl]prop-2-enamide
Benzamidas	MS-275	Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl) carbamoyl]phenyl] methyl] carbamate
Tetrapeptideos ciclicos	Romidepsin ou depsipeptideo (FK-228)	(1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19, 22-pentone

HDIs, incluindo os derivados do butirato como o butirato sódico (NaB) (Figura 2), estão em evidencia como uma potencial nova classe de agentes antineoplásicos. Isto se deve ao fato destas substâncias promoverem ou auxiliarem uma variedade de diferentes mecanismos anti-câncer, como apoptose, parada do ciclo celular e diferenciação celular. Ensaios clínicos recentes têm demonstrado que a hiperacetilação de histonas pode ser realizada com segurança em humanos, e que o tratamento com estes agentes é plausível (Vernia et al., 2000; Bug et al., 2005; Byrd et al., 2005; Kuendgen et

al., 2005; Garcia-Manero et al., 2006; Giles et al., 2006; Kuendgen et al., 2006; Maslak et al., 2006; Blum et al., 2007; Gojo et al., 2007; Soriano et al., 2007; Garcia-Manero et al., 2008; Gimsing et al., 2008; Klimek et al., 2008). Muitos HDIs, incluindo Vorinostat (SAHA), depsipeptideo, MS-275, TSA e butirato sódico têm um efeito sinérgico no aumento da atividade antineoplásica de um grande número de fármacos quimioterápicos convencionais (Bolden et al., 2006; Glaser, 2007). Estes fármacos clássicos incluem gemcitabina, paclitaxel, cisplatina, etoposideo, e doxorubicina, que atuam sobre células malignas através de diferentes mecanismos (Fuino et al., 2003; Kim et al., 2003; Dowdy et al., 2006; Arnold et al., 2007; Rikiishi et al., 2007). Uma grande quantidade de estudos avaliando HDIs foram realizados nos últimos 5 anos, e mais de 100 patentes foram publicadas (Paris et al., 2008).

Figura 2. Fórmula molecular do butirato sódico (NaB).



HDIs: Uma Nova Alternativa no Tratamento Contra o Câncer

A utilização de HDIs em associação com fármacos antineoplásicos clássicos ou em combinação com outros agentes, como demetilantes de DNA, representa uma promissora alternativa de tratamento para o câncer. Estudos avaliaram, em diferentes linhagens celulares, os efeitos de HDIs e das

associações destes com diferentes agentes antineoplásicos clássicos na proliferação celular e apoptose (Kouraklis & Theocharis, 2006). Em leucemia diversos estudos foram realizados, avaliando combinações de diferentes HDIs com quimioterápicos clássicos, como: ácido retinóico, carboplatina, citarabina, cladribina, desatinib, doxorubicina, etoposideo, fludarabina, 4-hidroperoxiciclofosfamida, idarubicina, imatinib, 6-mercaptopurina, metotrexato, SN-38(metabólito ativo do irinotecano) e vincristina. Estes estudos apresentam resultados bastante animadores em relação a uma futura utilização de HDIs como adjuvantes no tratamento de leucemia (Nimmanapalli et al., 2003; Theocharis et al., 2003; Yu et al., 2003; Maggio et al., 2004; Fiskus et al., 2006; Sanchez-Gonzalez et al., 2006; Kano et al., 2007; Bartolini et al., 2008; Bouzar et al., 2009).

Especificamente em relação ao HDI butirato sódico (NaB), estudos avaliaram a utilização combinada deste HDI com diversos fármacos citotóxicos clássicos. Avaliando células de retinoblastoma Y79 e WERI-Rb1(Conway et al., 1998), verificou-se que o NaB combinado a vincristina aumentou significativamente a indução de apoptose. Sonnemann et al. (2006), avaliando diversos Inibidores de desacetilação de histona nas linhagens DAOY e UW228-2 de meduloblastoma, observaram que quando combinados à vincristina não promoveram aumento significativo na indução de morte celular. Em leucemias, o uso do HDI NaB combinado a agentes citotóxicos clássicos como uma alternativa em relação aos atuais tratamentos havia sido avaliado por apenas dois estudos. Siitonen et al. (2005), trabalhando com a linhagem celular de leucemia mieloblástica aguda OCI/AML-2, verificaram um grande aumento na citotoxicidade, quando butirato foi combinado com citarabina. Avaliando células

de leucemia THP-1, Ramos et al. (2004), verificaram que o NaB aumentou a apoptose induzida por citarabina e vincristina. Neste estudo concluiu-se que o butirato poderia ser um adjuvante promissor para o tratamento de leucemias em combinação com outros fármacos antineoplásicos clássicos. Até a realização do presente estudo, os efeitos da utilização combinada de fármacos citotóxicos clássicos e NaB ainda não haviam sido avaliados em LLA.

Objetivos

Objetivo Geral

- Avaliar os efeitos de fármacos antineoplásicos comumente utilizados para tratar leucemias, de NaB e da combinação destes fármacos com NaB na proliferação celular de células de leucemia, utilizando-se como modelo a linhagem celular humana T linfoblástica, Jurkat.

Objetivos Específicos

- Padronização do cultivo e determinação da curva de crescimento celular da linhagem de leucemia linfoblástica aguda humana Jurkat;
- Determinação das curvas de dose dos fármacos bleomicina, citarabina, doxorubicina, etoposideo, metotrexato, vincristina e do NaB na linhagem de leucemia linfoblástica aguda humana Jurkat;
- Avaliação dos efeitos de NaB, dos antineoplásicos bleomicina, citarabina, doxorubicina, etoposideo, metotrexato e vincristina, e do uso combinado combinados destes fármacos com NaB sobre a proliferação celular na linhagem de leucemia linfoblástica aguda humana Jurkat.

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Artigo Científico

Leukemia Research

Brief communication

Running head: Potentiation of cytotoxic effects with sodium butyrate in leukemia.

Running title: Sodium butyrate enhances the cytotoxic effect of antineoplastic drugs in human lymphoblastic T-cells.

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Abstract

Sodium butyrate (NaB), a potent histone deacetylase inhibitor, induces cell cycle arrest and apoptosis in malignant cells. We investigated the effects on cellular proliferation *in vitro* when combining NaB with antineoplastic drugs commonly used to treat leukemias. Our results demonstrate that NaB increases the cytotoxic effects of cytarabine and etoposide, but not of bleomycin, doxorubicin, vincristine or methotrexate. These data suggest that NaB is a promising adjuvant therapeutic agent for the treatment of lymphoblastic leukemias, and provides a basis for further studies in this field.

Keywords: Acute lymphoblastic leukemia, sodium butyrate, adjuvant therapy, chemotherapy.

Introduction

Acute lymphoblastic leukemia (ALL), a malignant disorder of lymphoid progenitor cells, affects both children and adults, with peak prevalence between the ages of 2 and 5 years. The drugs currently in use to treat leukemia were initially developed and tested between the 1950s and 1970s. Although the steady progress in the development of antineoplastic agents has lead to a cure rate of about 80% in children, these drugs are often used in high doses, causing several toxic side-effects. For this reason, efforts to identify new chemotherapeutic agents are still warranted [1].

The balance between the acetylation and deacetylation of histones plays a significant role in regulating eukaryotic gene expression. Histone deacetylase inhibitors (HDIs), like sodium butyrate (NaB), are in evidence as a potential new class of antineoplastic agents because they are able to promote or enhance a variety of different anticancer mechanisms, including apoptosis, cell cycle arrest and cellular differentiation. Clinical studies have shown that histone hyperacetylation can be achieved safely in humans, and that treatment with such agents is plausible. Therefore, the use of HDIs in association with classical chemotherapeutic drugs or in combination with DNA-demethylating agents could be a promising treatment alternative [2].

The effects of coadministering classical anticancer drugs and NaB have not yet been evaluated in ALL. In the present study, we evaluated the antiproliferative effects *in vitro* elicited by cotreatment of NaB and other anti-cancer drugs commonly used to treat leukemias on the human lymphoblastic cell line, Jurkat.

Materials and Methods

Cell Lines and Reagents

The human T-lymphoblastic cell line, Jurkat, was obtained from the American Type Culture Collection (Rockville, Maryland, USA) and grown in suspension in RPMI 1640 (Gibco BRL, Carlsbad, USA) containing 0.1% Fungizone and 100U/l gentamicin supplemented with 10% fetal bovine serum (FBS; Sorali, Campo Grande, Brazil) at 37°C in a humidified incubator under 5% CO_2. Sodium butyrate (NaB) was obtained from Sigma-Aldrich (St. Louis, MO, USA); bleomycin (Bleo) and etoposide (VP-16) from Bristol-Myers Squibb (São Paulo, Brazil); cytarabine (Ara-C), doxorubicin (Doxo) and vincristine (VCR) from Pfizer (São Paulo, Brazil); and methotrexate (MTX) from Mayne.

Cellular proliferation assay

Cells were counted and seeded at 10^6 cells/T25 flask in 5ml culture medium. Twenty-four hours later, cells were recounted (time 0) and treated with NaB (0.5mM; 1mM), vincristine (VCR 0.1nM; 0.5nM), cytarabine (Ara-C 50nM; 100nM), doxorubicin (Doxo 1.0nM; 50nM), etoposide (VP-16 200nM; 500nM), methotrexate (MTX 200nM; 500nM) and bleomycin (Bleo 1μU; 1.5μU) or a combination of each dose. At the end of the treatment period (48h), 10μl of cellular suspension was homogenized 1:1 with 0.4% trypan blue solution (Sigma-Aldrich). Cells were counted in a hemocytometer and viability was determined by the trypan blue exclusion test. Results are displayed as growth indexes, calculated using the following formula:

[(# of viable cells at 48h) – (# of viable cells at time 0)] / # of viable cells at time 0]

Statistical analysis

Results are representative of 3 independent experiments performed in triplicates, and are expressed as mean values ± standard deviation (SD) of independent experiments. Data were analyzed by one-way analysis of variance – ANOVA, followed by the Tukey-Kramer test, using the SPSS program, version 12.0. Values of $P<0.05$ were considered statistically significant.

Results

All treatments promoted a statistically significant reduction in cellular proliferation when compared to the control (Fig. 1, A-F). Regardless of the dose, cotreatment of NaB and VCR (Fig. 1A), Doxo (Fig. 1C), MTX (Fig. 1E) or Bleo (Fig. 1F) did not promote a significant reduction in proliferation when compared to either drug or NaB alone. On the other hand, Fig. 1B shows that cotreatment of NaB (0.5mM) and Ara-C (50nM) for 48h resulted in a statistically significant reduction in cellular proliferation when compared to monotreatment with NaB (0.5mM) ($P=0.0001$) or Ara-C (50nM) ($P=0.007$). Similarly, Fig. 1D shows that cotreatment with NaB (0.5mM) and VP-16 (200nM) promoted a statistically significant reduction in cell growth when compared to monotreatments with NaB (0.5mM) ($P=0.0001$) or VP-16 (200nM) ($P=0.0001$). It is of interest to note that

cotreatment of NaB (0.5mM) and Ara-C (50nM), and NaB (0.5mM) and VP-16 (200nM) resulted in the same antiproliferative effect induced by higher concentrations of Ara-C (100nM) (P=0.381) and VP-16 (500nM) (P=0.751), respectively.

Discussion

Our results demonstrate that treating a human lymphoblastic leukemia cell line with several chemotherapeutic agents in combination with the histone deacetylase inhibitor (HDI), sodium butyrate (NaB), potentiates the cytotoxic effects of some, but not all, anticancer drugs. Although the association between HDIs and classical anticancer drugs have been evaluated in solid tumors [3][4][5] and leukemias [6][7][8], the effects of these combinations have not yet been evaluated in acute lymphoblastic leukemia (ALL). Moreover, most studies regarding the cotreatment of classical chemotherapeutic agents and NaB were done so in order to evaluate apoptosis induction and gene expression modulation, leaving the question of whether there is an additive effect on cellular proliferation unanswered. In this study, the experiments were carefully designed to evaluate the effects on cellular proliferation *in vitro* upon cotreatment of antineoplastic drugs and NaB. Dose curves were performed to establish the lowest concentrations that significantly reduced cellular proliferation when compared to the control, albeit without producing significant cell death (data not shown). These doses, in nanomolar concentrations, differ from the ones frequently used to evaluate apoptosis, which are usually in the micromolar range. Administering these drugs in lower concentrations allowed us

to asses with greater ownership whether NaB does indeed augment the effects elicited by each drug.

At the doses tested, we observed that NaB (0.5mM) increases the cytotoxic effect of Ara-C (50nM) and of VP-16 (200nM), but not of Bleo (1.5uU), Doxo (1.0nM), VCR (0.1 and 0.5nM) and MTX (10nM). This result is relevant in that it demonstrates, for the first time to our knowledge, that NaB does not necessarily have an additive effect in reducing cell growth when given in combination with all the antineoplasic drugs tested in this panel. Kouraklis et al. (2006), in their review, conclude that the use of HDIs in association with classical chemotherapeutic agents represents a promising therapy for cancer patients. Our results suggest that cotreatmnet with HDIs is a viable alternative when combined with certain anticancer agents, but that often times the observed antiproliferative effects are due to NaB or the drug alone, rather than as a result of cotreatment. The results presented in this study provide a basis for evaluating the effects of cotreatment with NaB and other anticancer drugs in lower concentrations, in order to clearly observe which combinations are better suited for testing in clinical trials.

The chemotherapeutic agents tested in this study cause several unwanted side effects. Meanwhile, clinical studies have shown that histone hyperacetylation can be achieved safely in humans [2], and that treatment with such agents is plausible. In addition to verifying that NaB enhances the cytotoxic effect of Ara-C and VP-16, we also observed that the antiproliferative effects elicited by NaB (0.5mM) in combination with Ara-C (50nM) and NaB (0.5mM) in combination with VP-16 (200nM) were statistically the same

produced by Ara-C (100nM) and VP-16 (500nM) alone, respectively. This suggests that cotreatment with NaB and Ara-C or VP-16 might allow these drugs to be administered in smaller doses, all the while maintaining the same effect, consolidating the idea of utilizing NaB as a new anticancer adjuvant drug in order to decrease the toxic side-effects caused by current anticancer protocols. The clinical use of these cotreatments could reduce frequent problems, like dose reductions and temporary discontinuation of treatment as a result of toxicity, and thus could improve the treatment itself and the patient's quality of life.

Further investigations are needed to consolidate the use of NaB in antileukemia adjuvant therapy. Understanding the mechanisms by which NaB enhances the effects of one drug over another is also necessary in order to potentiate its effects and to develop similar drugs that display a broader range when used for adjuvant therapy.

5. Acknowledgments

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7. Figure 1

Figure 1 Legends: Effects of vincristine (VCR), cytarabine (Ara-C), doxorubicin (Doxo), etoposide (VP-16), methotrexate (MTX), and bleomycin (Bleo) with or without sodium butyrate (NaB) on cellular proliferation. Jurkat cells were treated for 48h with (A) VCR 0.1nM and 0.5nM, (B) Ara-C 50nM and 100nM, (C) Doxo 1.0nM and 50nM, (D) VP-16 200nM and 500nM, (E) MTX 10nM and 50nM and (F) Bleo 1.0μU and 1.5μU, with or without sodium butyrate (NaB) 0.5mM and 1.0mM. Growth index main values and standard deviations are shown. *P*-value was determined using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test.

*P<0.005, ** P<0.001. All treatments promoted a statistically significant reduction in cell growth when compared to the control.

Considerações Gerais

Os resultados obtidos demonstram que o tratamento de uma linhagem celular de leucemia linfoblástica com diversos agentes quimioterápicos em combinação com o inibidor de desacetilação de histona (HDI), butirato sódico (NaB), potencia os efeitos citotóxicos de alguns, mas não de todos fármacos antineoplásico. Apesar da associação entre HDIs e fármacos citotóxicos clássicos terem sido avaliadas em tumores sólidos e leucemias, os efeitos destas combinações ainda não haviam sido avaliados em leucemia linfoblástica aguda (LLA). Além disso, a maioria dos estudos avaliando cotratamentos de agentes quimioterápicos clássicos e NaB foram desenhados com o intuito de avaliar indução de apoptose e modulação da expressão gênica, não avaliando diretamente a questão em relação a efeitos combinados de substancias na redução de proliferação celular. Neste estudo, os experimentos foram cuidadosamente desenhados para se avaliar os efeitos dos cotratamentos com fármacos antineoplásicos e NaB na proliferação celular *in vitro*. Curvas de doses foram realizadas para se estabelecer as menores concentrações que significativamente reduziram a proliferação celular quando comparadas ao controle, mas sem produzirem significante morte celular. Estas doses, em nano molar, diferem das freqüentemente usadas para avaliar apoptose, que são geralmente na ordem de micro molar. Administrar estas drogas em concentrações menores nos permitiu avaliar com maior propriedade se NaB efetivamente aumenta os efeitos produzidos por cada um desses fármacos clássicos.

Nas doses testadas, nos observamos que NaB (0.5mM) aumenta o efeito citotóxico de Ara-C (50nM) e de VP-16 (200nM), mas não de Bleo (1.5uU), Doxo (1.0nM), VCR (0.1 e 0.5nM) e MTX (10nM). Este resultado é relevante, pois demonstra, pela primeira vez, que NaB não necessariamente promove um efeito aditivo, na redução do crescimento celular, quando combinado com qualquer fármaco antineoplásico. Kouraklis et al. (2006), em sua revisão, conclui que o uso de HDIs em associação com agentes quimioterápicos clássicos representa uma promessa no tratamento do câncer. Nossos resultados sugerem que o cotratamento com HDIs é uma alternativa viável quando combinados com alguns agentes anti-câncer, entretanto as vezes os efeitos antiproliferativos observados são devidos ao NaB ou fármaco isoladamente, e não como resultado de cotratamento. Os resultados apresentados neste estudo proporcionam as bases para futuras avaliações dos efeitos de cotratamentos com NaB e outros fármacos anti-câncer em menores concentrações, a fim de se observar claramente quais combinações são mais adequadas para serem testados em ensaios clínicos.

Os agentes quimioterápicos testados neste estudo causam diversos efeitos colaterais indesejados. Entretanto, ensaios clínicos recentes têm demonstrado que a hiperacetilação de histonas pode ser realizada com segurança em humanos, e que o tratamento com estes agentes é plausível [2]. Além de verificar que a NaB aumenta o efeito citotóxico de Ara-C e VP-16, observamos também que os efeitos antiproliferativos produzidos pelo NaB (0,5 mm) em combinação com Ara-C (50nM) e NaB (0,5 mm) em combinação com VP-16 (200nM) foram estatisticamente os mesmos produzidos pelo tratamento isolado com Ara-C (100 nM) e VP-16 (500nM), respectivamente. Isso sugere

que o cotratamento de NaB com Ara-C ou VP-16 poderia permitir que estes medicamentos fossem administrados em doses menores, consolidando a idéia de utilizar NaB como um novo fármaco antineoplásico adjuvante, a fim de diminuir os efeitos colaterais e tóxicos causados pelos atuais protocolos antineoplásicos. O uso clínico destas combinações poderia reduzir problemas freqüentes, como reduções de doses e descontinuação temporária do tratamento como resultado de toxicidade, e, assim, poderia melhorar o tratamento em si e a qualidade de vida do paciente.

Novas investigações são necessárias para consolidar o uso de NaB na terapia adjuvante antileucemia. Compreender os mecanismos pelos quais NaB potencia os efeitos de determinados fármacos e não de outros também é necessário, a fim de potenciar os seus efeitos e desenvolver medicamentos similares cada vez melhores.

Anexo 1

Accepted article entitled Sodium butyrate enhances the cytotoxic effect of antineoplastic drugs in human lymphoblastic T-cells. Published in *Leukemia Research,* Volume 33, Issue 2, February 2009, Pages 218-221.

Anexo 2

Review article submitted for publication entitled "Histone deacetylase inhibitors: A new perspective for the treatment of leukemia," submitted to the journal Advances in Hematology on February 13th, 2009 (assigned the number AH/347656).

Advances in Hematology

Review article

Running head: Histone deacetylase inhibitors for treating leukemia.

Running title: Histone deacetylase inhibitors: A new perspective for the treatment of leukemia

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Abstract

Histone deacetylase inhibitors (HDIs) promote or enhance several different anticancer mechanisms and therefore are in evidence as potential antileukemia agents. Studies on leukemia have provided examples for their functional implications in cancer development and progression, as well as their relevance for therapeutic targeting. A number of HDIs have been tested in clinical trials, and most studies have shown that they are safe, all the while demonstrating significant clinical activity. The use of HDIs in association with other molecules, such as classical chemotherapeutic drugs and DNA demethylating agents, have been implied as a promising treatment alternative for leukemia patients, so much so that the clinical use of HDIs for treating these patients will be a reality in a short time. Further clinical trials involving a broader number of HDIs used either alone or in combination with other agents are needed to consolidate the use of these epigenetic modulators on leukemia therapy.

Keywords: leukemia, epigenetics, histone deacetylase inhibitors, clinical trials

Introduction

Leukemia, a malignant disorder of blood progenitor cells that affects both children and adults, is currently treated with drugs that were initially developed and tested between the 1950s and 1970s. Although the steady progress in the development of effective treatments has lead to a cure rate of about 80% in patients with acute lymphoblastic leukemia (ALL), the cure rates for other types of leukemia remain much lower. Moreover, the drugs most frequently used to treat this malignancy are often administered in high doses, causing several toxic side effects. For this reason, efforts to identify new chemotherapeutic or adjuvant agents are still warranted [1].

Epigenetic alterations, such as the deacetylation of histones, are known to contribute to cellular transformation by silencing critical genes [2]. Histone deacetylase inhibitors (HDIs) promote or enhance several different anticancer mechanisms, such as apoptosis, cell cycle arrest and cellular differentiation, and therefore are in evidence as potential adjuvants [3,4]. Studies on leukemia have provided examples for their functional implications in cancer development and progression, as well as their relevance for therapeutic targeting [5]. A number of HDIs are currently undergoing clinical trials, owing to their ability to revert some of the aberrant epigenetic states associated with cancer. These studies have shown that histone hyperacetylation can be achieved safely in humans, and that treatment with such agents is plausible [6,7].

The use of HDIs alone or in association with others molecules, such as classical chemotherapeutic drugs and DNA demethylating agents, could be a promising treatment alternative for leukemia patients. Here we review the main

studies concerning the use of histone deacetylase inhibitors in treating the different types of leukemia.

Current Treatment Options for Leukemia

Leukemia is a malignant cancer of the bone marrow and blood that affects both children and adults, and is commonly divided into four categories: acute myelogenous (AML) or chronic myelogenous (CML), involving the myeloid elements of the bone marrow (leukocytes, erithrocytes and megakaryocytes) and acute lymphoblastic (ALL) or chronic lymphoblastic (CLL), involving the cells of the lymphoid lineage. Standard treatment for all types of leukemia usually involves chemotherapy and/or bone marrow transplantation and/or radiation therapy.

The drugs currently in use to treat leukemia were initially developed and tested between the 1950s and 1970s [1]. The main classical drugs that are now being used in the clinic are asparaginase, cyclophosphamide, cytarabine, daunorubicin, doxorubicin, etoposide, mercaptopurine, methotrexate, mitoxantrone, thioguanine and vincristine (Table 1), being that treatment usually involves a combination of two or more anticancer drugs.

Table 1. Classical drugs currently in use to treat leukemia and their mechanism of action. Drug class is listed in italics, followed by the medication name in bold.

Mechanism of Action			**Classical drugs to treat leukemia**
Spindle Poison / Mitotic Inhibitor (M phase)	Block microtubule assembly		*Vinca alkaloids*: **Vincristine**
DNA replication inhibitor	DNA precursors/ antimetabolites (S phase)	Folic acid	*Dihydrofolate reductase inhibitor*: **Methotrexate**
		Purine	*Thiopurine*: **Mercaptopurine**
			Halogenated/ribonucleotide reductase inhibitors: **Cladribine, Clofarabine, Fludarabine**
			Thiopurine: **Thioguanine**
		Pyrimidine	*DNA polymerase inhibitor*: **Cytarabine**
		Deoxyribonucleotide	*Ribonucleotide reductase inhibitor*: **Hydroxyurea**
	Topoisomerase inhibitor (S phase)	II	*Podophyllum*: **Etoposide, Teniposide**
		II+Intercalation	*Anthracyclines*: **Daunorubicin, Doxorubicin, Idarubicin**
			Anthracenediones: **Mitoxantrone**
	Crosslinking of DNA (CCNS)	Alkylating	*Nitrogen mustards*: **Mechlorethamine, Cyclophosphamide, Chlorambucil.**
			Alkyl sulfonates: **Busulfan**
		Alkylating-like	*Platinum*: **Carboplatin, Cisplatin**
Other	Enzyme inhibitors		*Prl*: **Bortezomib**
	Other/ungrouped		*Retinoids:* **Tretinoin**
			Asparagine depleter: **Asparaginase**
			Vorinostat

Owing to the tremendous clinical variability among remissions observed in leukemic patients, the treatment for leukemia is very complex. Patients who are resistant to therapy have very short survival times, regardless of when the resistance occurs. Moreover, the drugs most frequently used to treat this malignancy are often administered in high doses, causing several toxic side effects. Despite improvements in outcome with current treatment programs, efforts to identify new chemotherapeutic or adjuvant agents for the treatment of all types of leukemia are still warranted.

Histone Deacetylases (HDACs) and Histone Deacetylase Inhibitors (HDIs)

Chromatin organization is crucial for the regulation of gene expression. In particular, both the nucleosome properties and positioning influence promoter-specific transcription in response to extracellular or intracellular signals. The acetylation and deacetylation of histones play significant roles in transcriptional regulation of eukaryotic cells, and are catalyzed by specific enzyme families: histone acetyl-transferases (HATs) and histone deacetylases (HDACs), respectively. HATs were originally identified as transcriptional co-activators and HDACs as yeast transcriptional regulators [8]. HDACs are a family of enzymes present in bacteria, fungi, plants, and animals that remove the acetyl moiety from the ϵ-amino groups of the lysine residues present within the N-terminal extension of the core histones. Consequently, the positive charge density on the N-termini of the core histones increases, strengthening the interaction with the negatively charged DNA and blocking the access of the transcriptional machinery to the DNA template [7].

The balance between acetylation and deacetylation is an important factor in regulating gene expression, and is thus linked to the control of cell fate. Disruption of HAT or HDAC activity is possibly associated with cancer development [9], and if so the molecular processes leading to the activation or repression of transcription are possible targets for anticancer therapy. HDACs have been implicated for the first time in cancer while studying acute promyelocytic leukemia [10]. Since then, HDAC silencing or inhibition has been shown to have an impact on cell cycle, cell growth, chromatin decondensation, cell differentiation, apoptosis, and angiogenesis in several cancer cell types [7].

Since inhibition of HDAC activity reverses the epigenetic silencing frequently observed in cancer, various HDAC inhibitors (HDIs) have been developed for therapeutic purposes (Table 2). These include short-chain fatty acids (such as valproic acid and butyrates), hydroxamic acids (such as Vorinostat (SAHA), Trichostatin A (TSA), Panobinostat (LBH-589), Belinostat (PXD101) and tubacin), benzamides (MS-275), and cyclic tetrapeptides (such as trapoxin, apicidin and depsipeptide), as well as a variety of other chemical compounds [4,6,11-14]. HDAC inhibitors also have varying degrees of specificity, although the molecular anticancer mechanism of each specific agent is not completely clear. The creation of inhibitors with greater specificity will enable each HDAC function to be more fully elucidated, besides yielding improved efficacy and reduced toxicity [4]. Current research in cancer therapy focuses on the design of drugs that are specific against molecular alterations found only in the transformed cell. The aim is to associate a specific tumor type with a specific gene expression profile, thus defining the alteration responsible for each cancer [7].

Table 2. Main histone deacetylase inhibitors evaluated as adjuvants for the treatment of leukemia.

Class	Compound	IUPAC Name
Short-chain fatty acids	Valproic acid	2-propylpentanoic acid
	Butyrates	Butanoate
Hydroxamic acids	Vorinostat (SAHA)	N'-hydroxy-N-phenyloctanediamide
	Trichostatin A (TSA)	(2E,4E)-7-(4-dimethylaminophenyl)-N-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide
	Panobinostat (LBH-589)	(E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide
	Belinostat (PXD101)	(E)-N-hydroxy-3-[3 (phenylsulfamoyl) phenyl]prop-2-enamide
Benzamides	MS-275	Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl) carbamoyl]phenyl] methyl] carbamate
Cyclic tetrapeptide	Romidepsin or depsipeptide (FK-228)	(1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19, 22-pentone

HDIs have been used as a new class of anticancer agents in clinical trials, and have been studied extensively in the laboratory. Clinical studies have shown that histone hyperacetylation can be achieved safely in humans, and that treatment with such agents is plausible [6, 7]. Many HDIs, including Vorinostat (SAHA), depsipeptide, MS-275, and TSA have a synergistic effect in enhancing the anticancer activity of a large number of conventional chemotherapeutic drugs [15, 16]. These include gemcitabine, paclitaxel, cisplatin, etoposide, and doxorubicin, which target malignant cells through different mechanisms [17-21]. A large amount of work has been carried out in the past 5 years in the field of HDIs, and more than 100 patents claiming new chemical series have been published [7].

Evidence for HDIs as a Therapeutic Strategy

Oncogenic proteins can, at times, incorporate HDACs. For example, acute promyelocytic leukemia (APL) normally responds to retinoic acid (RA), which induces differentiation and growth arrest [22-25]. However, when the retinoic acid receptor (RAR) is expressed as a fusion protein together with the promyelocytic leukemia zinc finger (PLZF) domain, cells become resistant to RA. This occurs as a result of the association of RAR-PLZF with HDACs and the subsequent repression of genes that trigger differentiation and that are normally induced by RA. When patients with APL who are not responding to RA are treated with RA in combination with an HDAC inhibitor, however, the cellular responses to RA are restored.

A combination of drugs currently in use with an HDAC inhibitor may be highly beneficial when a tumor type is characterized by these fusion proteins. A combination of phenylbutyrate and all-trans retinoic acid (ATRA), for example, induced histone hyperacetylation and completed remission in a case of a highly resistant promyelocytic leukemia patient that relapsed only 7 months later [4,10,26]. ATRA induces complete remission in a high proportion of patients with APL, albeit most of these patients usually develop RA resistance and eventually relapse. In an attempt to mimic clinical conditions for the treatment of leukemia, a RA-resistant sub-clone of the human promyelocytic leukemia cell line, HL60 (HL60-R), was developed to study the anti-proliferative and pro-apoptotic effects of the retinoid IIF (6-OH-11-O-hydroxyphenantrene) in comparison with RA. Whether valproic acid (VPA), another HDI, could enhance sensitivity to retinoids in HL60-R cells was also evaluated. Finally, the effects of

IIF on the expression of multidrug resistance-associated protein 1 (MRP1) and P-glycoprotein (P-gp) was also studied. It was found that IIF strongly suppressed cellular proliferation (as measured by growth curves) and induced apoptosis (as measured by DNA fragmentation and Annexin V detection assays), while RA remained practically ineffective. The addition of VPA to IIF accentuated the anti-proliferative effects of IIF alone and increased apoptosis; the combination of VPA with RA only induced growth arrest. Moreover, IIF reduced transmembrane transporter expression, particularly of P-gp, as shown by western blotting. These results suggest that IIF may be useful in controlling the proliferation of RA-resistant leukemia, especially in combination with an HDAC inhibitor, such as VPA [27].

Their broad synergistic capacity indicates that HDIs are likely to lower the threshold for tumor cells to undergo apoptotic cell death triggered by other agents. This can explain how one class of agents triggers cell death synergistically with such a wide array of other anticancer drugs. Consistent with this idea, the HDAC inhibitor MS-275 has been reported to decrease the levels of anti-apoptotic molecules and increase the levels of pro-apoptotic molecules, promoting differentiation or apoptosis in leukemia cells of human origin [28]. One study explored the effect of MS-275 against a panel of leukemia cells of human origin, each with defined genetic alterations. With an IC_{50} of less than 1μM, as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, MS-275 significantly induced growth arrest in the AML cell line, MOLM13, and in the biphenotypic leukemia cell line, MV4-11, which both possess an internal tandem duplication mutation in the fms-like tyrosine kinase 3 (FLT3) gene (FLT3-ITD). Exposure of these cells to MS-275 decreased total

and phosphorylated levels of FLT3 protein, resulting in inactivation of its downstream signaling pathways (Akt, ERK, and STAT5). Further studies found that MS-275 induced acetylation of heat shock protein 90 (HSP90) in conjunction with ubiquitination of FLT3, leading to degradation of FLT3 proteins in these cells. This was abrogated by treatment with the proteasome inhibitor bortezomib, confirming that FLT3 was degraded via the ubiquitin/proteasome pathway. Moreover, this study found that further inhibition of MEK/ERK signaling potentiated the effects of MS-275 in these cells. Altogether, these results suggest that MS-275 may be useful in treating individuals who carry a mutation on the FLT3 gene [29].

Another recent study found that MS-275 blocked Akt/mammalian target of rapamycin (mTOR) signaling in AML HL60 and APL NB4 cell lines, as seen by decreased levels of phosphorylated Akt (p-Akt), p-p70 ribosomal S6 kinase (p70S6K) and p-S6K, assessed by western blot analysis. Interestingly, further inactivation of mTOR by the rapamycin analog RAD001 (everolimus) significantly enhanced MS-275-mediated growth arrest and apoptosis of these cells in parallel with enhanced upregulation of p27(kip1) and downregulation of c-Myc. RAD001 also improved the ability of MS-275 to differentiate HL60 and NB4 cells, as measured by the expression of CD11b cell surface antigens. Also of note, RAD001 increased the ability of MS-275 to activate a myeloid differentiation-related transcription factor, CCAAT enhancer-binding protein-epsilon, in association with enhanced acetylation of histone H3 on its promoter. Finally, RAD001 significantly augmented the effects of MS-275 in inhibiting the proliferation of HL60 tumor xenografts in nude mice, without adverse effects. Taken together, concomitant administration of an HDI and an mTOR inhibitor

could be a promising treatment strategy for individuals diagnosed with AML or APL [30].

A number of combination strategies with targeted agents in different leukemic cells have been proposed, such as treatment with SAHA and imatinib during the advanced phases of CML [31], *in vitro* treatment with SAHA and desatinib in primary imatinib-sensitive or imatinib-resistant CML [32], *in vitro* treatment with the nucleoside analogue fludarabine [33], *in vitro* treatment with the proteasome inhibitor bortezomib combined with SAHA [34] and *in vitro* treatment with SAHA and an HSP90 antagonist [35]. The HDIs SAHA, LBH-589 and butyrate also appear to enhance the anticancer activity of targeted agents, including imatinib (a protein-tyrosine kinase inhibitor that targets the Bcr-Abl tyrosine kinase, which is created by the Philadelphia chromosome abnormality in CML) and the HSP90 antagonist 17-allylamino-17-demethoxygeldanamycin (17-AAG) in human leukemia cells [31,35-37]. The HDI depsipeptide (FK228) may mediate its effects on imatinib-resistant CML cells [38].

Enhanced anticancer activity has been observed with various HDIs in combination with other transcriptional modulators. This synergistic approach has shown to reactivate epigenetically silenced genes, such as CDKN1A (p21) and CDKN2B (p15). On the flip side, a potential caveat of HDI combinations is their ability to induce the cdk inhibitor, p21. While induction of p21 is critical for HDI-mediated cell-cycle arrest, it may also interfere with the efficient execution of the apoptotic cascade [10]. Therefore, agents that suppress the induction of p21 and that are stimulated by HDIs are highly desired, and several have been identified to date. Flavopiridol, LY294002, PKC412, and sorafenib have been reported to block HDI-mediated induction of p21, which increases the overall

apoptotic capacity and therefore contributes to their enhanced anticancer activity in human leukemia cells [39-42]. In addition to decreasing the levels of these anti-apoptotic proteins, several HDIs may also increase the expression of death receptors, such as DR5, and enhance the formation of the death-inducing signaling complex (DISC). In human acute leukemia cells, co-treatment with the HDI LAQ824, a cinnamic acid hydroxamate, enhances Apo-2L/TRAIL-induced death by enhancing several signaling cascades and apoptosis. [43]. The HDIs SAHA and butyrate induce apoptosis synergistically with the proteasome inhibitor bortezomib. The combination proteasome/HDI may represent a novel strategy for treating leukemias, including those that are apoptosis-resistant and Bcr/Abl-positive [34]. Dai et al. (2008) characterized the interactions between bortezomib and the clinically relevant HDIs romidepsin or belinostat in CLL cell lines. Co-administration of romidepsin or belinostat with bortezomib induced cell death synergistically in CLL cells, likely through mechanisms involving, among other factors, NF-κB inactivation and changes in the expression levels of pro-apoptotic and anti-apoptotic proteins. This study calls further attention to the strategy of combining HDI with proteasome inhibitors for treating CLL [44].

Interactions between the Bcr/Abl and aurora kinase inhibitor, MK-0457, and the HDI vorinostat were examined in Bcr/Abl(+) leukemia cells resistant to imatinib mesylate (IM), particularly those with the T315I mutation. Co-administration of vorinostat dramatically increased MK-0457-induced lethality in the K562 and LAMA84 cell lines. Notably, the MK-0457/vorinostat regimen was highly active against primary CD34(+) CML cells and against Ba/F3 cells bearing various Bcr/Abl mutations, such as T315I, E255K, and M351T, and against IM-resistant K562 cells exhibiting Bcr/Abl-independent, Lyn-dependent

resistance. These events were associated with inactivation and down-regulation of wild-type (wt) and mutated Bcr/Abl, particularly T315I. Treatment with MK-0457 resulted in an accumulation of cells with a DNA content of 4n or more. Co-administration of vorinostat and MK-0457, nonetheless, preferentially killed polyploid cells and markedly enhanced aurora kinase inhibition. Furthermore, vorinostat interacted with a selective inhibitor of aurora kinase A and B to potentiate apoptosis without modifying Bcr/Abl activity. Finally, vorinostat induced Bcl-2-interacting mediator of cell death (Bim) expression significantly, while blockade of Bim induction by siRNA dramatically diminished vorinostat´s ability to potentiate MK-0457-induced cell death. Together, these findings indicate that vorinostat strikingly increases MK-0457 activity against IM-sensitive and -resistant CML cells through inactivation of Bcr/Abl and aurora kinases, as well as by induction of Bim [45].

Fiskus et al. (2006) evaluated the combined effects of the novel tyrosine kinase inhibitor, AMN107, and the HDI LBH589 against human leukemia cells that are sensitive or not to IM and that express Bcr/Abl. As compared with either agent alone, co-treatment with AMN107 and LBH589 induced a pronounced reduction of viable cells in primary IM-resistant cells [46]. The mechanism by which LBH589 functions was investigated in Philadelphia chromosome-negative (Ph(-)) ALL. Two human Ph(-) ALL cell lines (T-cell MOLT-4 and pre-B-cell Reh) were treated with LBH589 and evaluated for biologic and gene expression responses. Nanomolar concentrations (IC_{50}: 5-20nM) of LBH589 induced cell-cycle arrest, apoptosis, and histone (H3K9 and H4K8) hyperacetylation. LBH589 treatment also increased mRNA levels of pro-apoptotic, growth arrest, and DNA damage repair genes such as FANCG, FOXO3A, GADD45A,

GADD45B, and GADD45G, being that the latter was the most over-expressed gene (up to a 45-fold induction) post-treatment. LBH589 treatment was associated with increased histone acetylation at the GADD45G promoter and with phosphorylation of histone H2A.X. This treatment was active against cultured primary Ph(-) ALL cells, including those from a relapsed patient, inducing a decrease in cell viability of up to 70% and increasing GADD45G mRNA expression up to 35-fold. Thus, LBH589 demonstrates considerable growth inhibitory activity against Ph(-) ALL cells, which is associated with up-regulation of genes that are critical for DNA damage response and growth arrest, and provides a rationale for exploring the clinical activity of LBH589 in treating patients with Ph(-) ALL [47].

Odenike et al. (2008) hypothesized that the HDI romidepsin could revert transcriptional repression, up-regulate specific target genes in AML, and differentiate the leukemic clone. The primary objectives of the study were to evaluate the safety and efficacy of romidepsin in advanced AML. Romidepsin has differential anti-leukemia and molecular activity in core binding factor AML. The authors suggest that the development of this agent in core binding factor AML should focus on combinations that target related mechanisms of gene silencing, such as DNA methylation [48].

There is no accepted curative therapy for adult T-cell leukemia (ATL). Depsipeptide has demonstrated major anti-tumor effects in leukemias and lymphomas. In one study, Chen et al. (2008) investigated the therapeutic efficacy of depsipeptide alone and in combination with daclizumab (HAT, humanized anti-Tac) in a murine model of human ATL. Combination of depsipeptide with daclizumab dramatically enhanced its anti-tumor effects, as

shown by both sIL-2R-alpha levels and survival of the leukemia-bearing mice when compared to those in the depsipeptide or daclizumab group alone. The authors concluded that therapeutic efficacy was significantly improved by combining depsipeptide with daclizumab, and provided a basis for future clinical trials that explore this combination for the treatment of ATL [49].

Some studies have evaluated the combination treatments with HDIs and classical chemotherapeutic drugs for treating leukemia. Combination treatment with the HDI butyrate and antineoplastic agents such as cytarabine (Ara-C), etoposide and vincristine was evaluated on the leukemic cell line THP-1. Butyrate increased apoptosis induced by the three agents as seen by measurement of DNA content, annexin exposure and morphological characteristics. This study concluded that butyrate could be a promising adjuvant for treating leukemia in combination with other antineoplastic drugs [50]. Similarly, another study investigated the effects of VPA and butyrate in an AML cell line, OCI/AML-2. Cytotoxicity, cell-cycle profiles and expression of cell-cycle regulating proteins were evaluated in the presence of Ara-C or etoposide. As single agents, VPA and butyrate inhibited AML cell growth, but did not induce cell death significantly. A dramatic increase in cytotoxicity was observed when combining VPA or butyrate with Ara-C, whereas co-addition of either one with etoposide had a modest effect on cell death [51]. Sanchez-Gonzalez et al. (2006) studied the cellular and molecular effects of combining the anthracycline idarubicin with two different HDIs: vorinostat (SAHA) and valproic acid (VPA). Their results indicate that the combination of an anthracycline with an HDI displays a synergistic effect *in vitro* [52].

Drug toxicity and resistance to chemotherapy are two major concerns when treating CLL with purine nucleoside analogues (PNA, i.e. fludarabine and cladribine). Bouzar et al. (2009) hypothesized that targeting epigenetic changes might address these issues, and to prove so evaluated the effects of the HDI VPA at clinically relevant concentrations. The data indicates that VPA may improve the outcome of PNA-based therapeutic protocols and provides a potential treatment alternative for both relapsed and front-line resistant patients, and for patients with high risk profiles [53].

The cytotoxic interaction of the HDI Romidepsin (FK228) in combination with conventional antileukemic agents was evaluated using several human leukemia cell lines. FK228 demonstrated an additive effect with Ara-C, carboplatin, doxorubicin, etoposide, 4-hydroperoxy-cyclophosphamide, 6-mercaptopurine and SN-38 (an active metabolite of irinotecan) in all the cell lines studied. FK228 in combination with methotrexate had an antagonistic effect in three of the four cells lines, whereas the combination of FK228 and vincristine behaved similarly in only one of the four cell lines. An additive effect was observed when FK228 was co-administered with imatinib in all three Ph(+) leukemia cell lines. These findings suggest that FK228 is a promising adjuvant candidate, except when co-administered with methotrexate and vincristine [54].

A recent study investigated the effects on *in vitro* cellular proliferation when combining sodium butyrate (NaB) with antineoplastic drugs commonly used to treat leukemias. NaB increased the cytotoxic effects of Ara-C and etoposide, but not of bleomycin, doxorubicin, vincristine or methotrexate. These data suggests that NaB is a promising adjuvant therapeutic agent for the treatment of lymphoblastic leukemias, and provides a basis for further studies in

this field [55]. Antileukemia activity has also been evaluated with HDIs are administered in combination hypomethylating agents. The combination of 5-aza-2'-deoxycytidine (DAC), a hypomethylating agent with significant antileukemia activity in humans, with VPA had a synergistic effect in growth inhibition, induction of apoptosis, and reactivation of p57KIP2 and p21CIP1 on human leukemia cell lines, suggesting that the combination of DAC and VPA could have significant antileukemia activity *in vivo* [56]. The use of HDIs alone has also been evaluated. The *in vitro* effect of a new hydroxamate derivative, ITF2357, was tested using an AML cell line as a model. This compound showed selective low-dose anti-leukemic activity on AML1/ETO-positive cells, emerging as a potent therapeutic agent, particularly in AML1/ETO-positive cells [57].

FR235222, a novel HDI, triggered accumulation of acetylated histone H4, inhibition of cellular proliferation and G1 cell-cycle arrest, accompanied by an increase in p21 protein levels and a down-regulation of cyclin E in the human promyelocytic leukemia cell line, U937. At a concentration of 50nM, the compound was also able to increase both mRNA and protein levels of annexin A1 (ANXA1) without affecting apoptosis. Similar effects were observed in the human CML cell line, K562, and in the human T-cell leukemia cell line, Jurkat. Cell-cycle arrest and ANXA1 expression were also induced by different HDIs like suberoylanilide hydroxamic acid (SAHA) and trichostatin-A (TSA). FR235222, when used at 0.5μM, triggered apoptosis in all leukemia cell lines, an event associated with an increased expression of the full-length (37kDa) ANXA1 protein and the appearance of a 33kDa N-terminal cleavage product in both the cytosol and membrane. These results suggest that ANXA1 expression may mediate cell-cycle arrest induced by low doses of FR235222, whereas

apoptosis induced by high doses of FR235222 is associated with ANXA1 processing [58].

Malignant myeloblasts arising in high-risk myelodysplastic syndrome (MDS) and AML are both characterized by the constitutive activation of the anti-apoptotic transcription factor, NF-κB. Fabre et al. (2008) found that DNA methyltransferase (DNMT) inhibitors, such as azacytidine and 5-aza-2'-deoxycytidine, and HDIs, such as trichostatin A and valproic acid, efficiently induces apoptosis in the P39 MDS/AML cell line, correlating with an inhibition of NF-κB (which translocated from the nucleus to the cytoplasm). This effect was obtained rapidly, within a few hours of treatment, suggesting that it was not due to epigenetic reprogramming. Indeed, DNMT and HDIs decreased phosphorylated levels of the NF-κB upstream regulator, IKKα/β, an effect that was also observed in enucleated cells. This was corroborated by the fact that circulating myeloblasts from AML patients treated with the DNMT inhibitor 5-aza-2'-deoxycytidine manifested a rapid (2 hours post-treatment) inhibition of NF-κB and IKKα/β. Therefore, it is possible that DNMT and HDIs inhibit the constitutive activation of NF-κB in malignant myeloblasts *in vitro* and *in vivo* through a novel signaling mechanism [59].

Rosato et al. (2008) evaluated the role of reactive oxygen species (ROS) on DNA damage and potentiation of fludarabine-mediated cell death by the HDI LAQ-824 in human leukemia cells. Addition of fludarabine, an antimetabolite used in the treatment of CLL, potentiated DNA damage, which was incompatible with cell survival, and triggered multiple pro-apoptotic signals, including activation of nuclear caspase-2 and release of histone H1.2 into the cytoplasm. The latter event induced activation of Bak and culminated in

pronounced mitochondrial injury and apoptosis. These findings provide a mechanistic basis for understanding the role of early HDI-induced ROS generation and modulation of DNA repair processes in potentiating nucleoside analogue-mediated DNA damage and lethality in leukemia. Moreover, they show, for the first time, the link between HDI-mediated ROS generation and the recently reported DNA damage observed in cells exposed to these agents [60].

Lu et al. (2008) found that the ethanol extract from wild fruiting bodies of *Antrodia camphorata* (EEAC) could induce apoptosis in HL60 cells via histone hypoacetylation, up-regulation of histone deacetyltransferase 1 (HDAC 1), and down-regulation of histone acetyltransferase genes, including GCN 5, CBP and PCAF, in a dose-dependent manner. Combined treatment of 100nM TSA and 100μg/ml EEAC elicited a synergistic inhibition of cell growth and an increase in apoptosis. EEAC effectively increased the cytotoxic sensitivity of TSA through the up-regulation of DR5 and activation of NF-κB. In this study, bioassay-guided fractionation of EEAC showed that the major active compound, zhankuic acid A, was also a bioactive marker. These findings present an experimental basis for developing EEAC as a potential chemotherapeutic adjuvant [61].

Current Preclinical and Clinical Trials of HDIs in Patients with Leukemia

Preclinical studies with the HDI depsipeptide (FK228) in CLL and AML have demonstrated that it effectively induces apoptosis at concentrations in which HDAC inhibition occurs. Byrd et al. (2005) carried out a phase 1 and pharmacodynamic study of depsipeptide (FK228), in which patients with CLL or AML were treated with depsipeptide intravenously. Neither life-threatening

toxicities nor cardiac toxicities were noted, although the majority of patients experienced progressive fatigue, nausea, and other constitutional symptoms that prevented repeated dosing. Several patients had evidence of antitumor activity following treatment, but no partial or complete responses were noted. An increase in HDAC inhibition and histone acetylation of at least 100% was noted, as well as an increase in p21 promoter H4 acetylation, p21 protein levels, and 1D10 antigen expression. The researchers concluded that depsipeptide effectively inhibits HDAC *in vivo* in patients with CLL and AML, but its use in the current schedule of administration is limited by progressive constitutional symptoms [62]. A recent study evaluated the toxicity, pharmacokinetic profile, and selected pharmacodynamic properties of depsipeptide in patients with AML or MDS. The most common grade 3/4 toxicities were febrile neutropenia/infection, neutropenia/thrombocytopenia, nausea, and asymptomatic hypophosphatemia. No clinically significant cardiac toxicity was observed. The responses seen in all eleven examined patients were: one patient with complete remission, six patients with stable disease, and progression of disease in four patients. Exploratory laboratory studies showed modest but rapid increases in apoptosis and changes in myeloid maturation marker expression. The authors concluded that depsipeptide therapy can be administered with acceptable short-term toxicity. However, gastrointestinal symptoms and fatigue seem to be treatment-limiting after multiple cycles. Depsipeptide monotherapy has limited clinical activity in unselected AML/MDS patients [75].

A phase 2 study of valproic acid (VPA) alone or in combination with all-trans retinoic acid (ATRA) was carried out in patients with MDS and in relapsed

or refractory AML patients. ATRA exerted no additional effects in patients receiving the combination from the start, nor did it benefit primary VPA non-responders. However, of ten VPA responders who relapsed, four achieved a second response after addition of ATRA. Kuendgen et al. (2005) concluded that VPA is clinically useful in low-risk MDS. For patients with high-risk MDS, VPA may be combined with chemotherapy or demethylating drugs. If patients relapse after an initial response to VPA, ATRA has the potential to induce a prolonged second response [63]. Similarly, VPA and ATRA have been evaluated clinically in patients with AML. Biological activity of VPA was confirmed by serial analysis of HDAC2 protein levels in peripheral blood (PB) mononuclear cells. Treatment with VPA/ATRA resulted in transient disease control in a subset of patients with AML who evolved from a myeloproliferative disorder, but not in patients with primary or MDS-related AML [64]. Another study also evaluated the use of VPA alone or in combination with ATRA in AML patients. VPA was administered to AML patients who were of advanced age and/or medically unfit to receive intensive chemotherapy. The response rate was only 5% according to the International Working Group (IWG) criteria for AML but achieved 16% when IWG response criteria for MDS, which capture hematogenic improvement and stabilization of the disease, were used. These endpoints, which were not necessarily correlated with diminishing blast counts, were relevant for the patients' quality of life. Among the patients with a peripheral blast count below 5%, six (26%) showed a diminishing blast count, and five of these had a complete peripheral blast clearance. The authors concluded that VPA had beneficial effects, but is not sufficiently active to be employed alone as therapy for treating AML. In elderly patients who are

medically unfit for standard chemotherapy, VPA may be combined with pharmacologic inhibitors of pathways that are involved in the pathogenesis of AML, such as FLT3- or FTI-inhibitors, or with demethylating agents [67].

In a phase I study, LBH589 was administered intravenously in patients with AML, ALL, or MDS. The levels of histone acetylation were measured using quantitative flow cytometry, and plasma LBH589 concentrations were also assayed. Four dose-limiting toxicities were observed. Other potentially LBH589-related toxicities included nausea (40%), diarrhea (33%), vomiting (33%), hypokalemia (27%), loss of appetite (13%), and thrombocytopenia (13%). The area under the curve increased proportionally with the dose and exhibited a terminal half-life of approximately 11 hours. The intravenous administration of LBH589 was well tolerated at doses below 11.5 mg/m^2, with consistent transient biological and anti-tumor effects [65].

A pilot study was designed to target DNA methylation and histone deacetylation through the sequential administration of 5-azacytidine followed by sodium phenylbutyrate (PB) in patients with AML or MDS. Fifty percent of patients were able to achieve a beneficial clinical response (partial remission or stable disease). One patient with MDS proceeded to allogeneic stem cell transplantation and is alive without evidence of disease 39 months later. The combination regimen was well tolerated, with common toxicities of injection site skin reaction (90% of the patients) from 5-azacytidine, and somnolence/fatigue from the sodium PB infusion (80% of the patients). Correlative laboratory studies demonstrated the consistent reacetylation of histone H4, although no relationship with the clinical response could be demonstrated. Results from this pilot study demonstrate that a combination approach targeting different

mechanisms of transcriptional modulation is clinically feasible with acceptable toxicity and measurable biologic and clinical outcomes [66]. Soriano et al. (2007) conducted a phase 1/2 study to evaluate the combination of 5-azacitidine (5-AZA), VPA, and ATRA in patients with AML or high-risk MDS. A significant decrease in global DNA methylation and induction of histone acetylation were achieved, and VPA blood levels were elevated in responders ($P < .005$). In conclusion, the combination proved to be safe and showed significant clinical activity [71].

Garcia-Manero et al. (2006) conducted a phase 1/2 study to evaluate the combination of 5-aza-2'-deoxycytidine (decitabine) and VPA in patients with advanced leukemia, including older, untreated patients. The patients were treated with decitabine administered concomitantly with escalating doses of VPA. Twelve (22%) patients had objective responses, including ten (19%) complete remissions (CRs) and two (3%) CRs with incomplete platelet recovery (CRp). Among ten elderly patients with AML or MDS, five (50%) responded (4CRs, 1CRp's). Induction mortality was observed in one (2%) patient. Major cytogenetic responses were documented in six of eight responders. Remission duration was of 7.2 months (range, 1.3-12.6+ months). Overall survival was of 15.3 months (range, 4.6-20.2+ months) in responders. Transient DNA hypomethylation and global histone H3 and H4 acetylation were induced, and were associated with p15 reactivation. Patients with lower pre-treatment levels of p15 methylation had a significantly higher response rate. In this work, the combination of epigenetic therapy was safe and active for treating leukemia, and was associated with transient reversal of aberrant epigenetic marks [68]. Another phase I study of decitabine alone or in combination with VPA in AML

determined an optimal biologic dose (OBD) of decitabine as a single agent and then the maximum-tolerated dose (MTD) of VPA combined with decitabine. Clinical responses were similar for decitabine alone or with VPA. Low-dose decitabine was safe and showed encouraging clinical and biologic activity in AML, but the addition of VPA led to encephalopathy at relatively low doses. The authors suggest that additional studies of decitabine alone or with an alternative deacetylating agent are warranted [70].

MS-275 is a benzamide derivative with potent HDAC inhibitory and anti-tumor activity in preclinical models. A phase 1 and pharmacologic study of MS-275 was carried out in adults with refractory and relapsed acute leukemias. Dose-limiting toxicities (DLTs) included infections and neurologic toxicity manifesting as unsteady gait and somnolence. Other frequent non-DLTs were fatigue, anorexia, nausea, vomiting, hypoalbuminemia, and hypocalcemia. Treatment with MS-275 also increased total and acetylated histone H3 and H4, p21 expression, and caspase-3 activation in bone marrow mononuclear cells. No responses by classical criteria were seen. The results demonstrated that MS-275 effectively inhibits HDAC *in vivo* in patients with advanced myeloid leukemias and should be further tested, preferably in patients with less advanced disease [69]. In 2007, Kuendgen & Gattermann published the review "Valproic acid for the treatment of myeloid malignancies". It was concluded that clinical trials with VPA have focused on AML and MDS, that VPA led to hematogenic improvement in a subset of patients when it was used alone or in combination with ATRA, which synergizes *in vitro*, and that, similarly to other HDIs, complete or partial remissions were rarely observed [72].

A Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes showed significant anti-leukemia activity at or below the MTD in patients with AML. Vorinostat effectively inhibited HDAC activity in peripheral blood and bone marrow blasts. Based on these results, the authors conclude that, even though the phase 1 and 2 trials of vorinostat alone or in combination regimens in AML/MDS are ongoing, further evaluation, including analysis of potentially predictive gene expression signatures for efficacy or resistance, is necessary [74].

In a phase 1 study in patients with leukemia or MDS, MGCD0103, an isotype-selective inhibitor of histone deacetylases targeted to isoforms 1, 2, 3, and 11, was administered orally three times weekly without interruption. The maximum tolerated dose was established at 60 mg/m^2, with dose-limiting toxicities (DLTs) presenting as fatigue, nausea, vomiting, and diarrhea at higher doses. Three patients achieved a complete bone marrow response (blasts below or equal to 5%). Pharmacokinetic analysis indicated absorption of MGCD0103 within one hour and an elimination half-life in plasma of 9 (+/- 2) hours. Exposure to MGCD0103 was proportional to doses up to 60 mg/m^2. Analysis of peripheral white cells demonstrated induction of histone acetylation and dose-dependent inhibition of HDAC enzyme activity. In summary, MGCD0103 is safe and demonstrates mechanism-based anti-tumor activity in patients with advanced leukemia [76]. MGCD-0103 is currently undergoing phase II clinical trials in patients with lymphoma, leukemia, myelodysplastic syndromes and solid tumors [73].

Gimsing et al. (2008) evaluated the safety, dose-limiting toxicity and maximum tolerated dose (MTD) of the novel hydroxamate histone deacetylase inhibitor belinostat (PXD101) in patients with advanced hematological neoplasms. The most common treatment-related adverse events (all grades) were nausea (50%), vomiting (31%), fatigue (31%) and flushing (31%). No grade 3 or 4 hematological toxicity as compared with baseline occurred, with the exception of grade 3 lymphopenia. Two related grade 4 adverse events of renal failure were observed. Both events occurred in patients with multiple myeloma and had similar characteristics, i.e. an acute episode of renal dysfunction (pre-existing nephropathy in one patient), with a metabolic profile and decrease in tumor burden consistent with tumor lysis syndrome. No other related grade 4 events were noted. The only related grade 3 events noticed in more than one patient were fatigue and neurological symptoms, being that one patient had status epilepticus in association with uremia and the other had paresthesia. All other related grade 3 events occurred as single events in patients, and no cardiac events were noted. No complete or partial remissions were noted in these heavily pre-treated (average of four prior regimens) patients. However, five patients, including two patients with diffuse large-cell lymphoma (including one patient with transformed CML, two patients with CLL, and one patient with multiple myeloma), achieved disease stabilization in two to nine treatment cycles. Therefore, intravenous belinostat at 600, 900 and 1000 $mg/m^2/d$ is well tolerated by patients with hematological malignancies. The study was carried out in parallel to a similar dose-finding study in patients with solid tumors, in which the MTD was determined to be 1000 $mg/m^2/d$ days 1-5 in

a 21-d cycle. This dose can also be recommended for phase II studies in patients with hematological neoplasms [77].

Conclusions

In vitro and *in vivo* studies have been carried out in recent years to evaluate the use of HDIs on leukemia treatments. A number of HDIs have been tested in clinical trials, demonstrating safe and significant clinical activity. The use of HDIs in association with others molecules, such as classical chemotherapeutic drugs and DNA demethylating agents, may be promising alternatives for treating patients with leukemia. The great potential of these epigenetic modulators for treating leukemia has provided the basis for many studies, including those designed to identify HDAC variants in order to predict drug response and composition, to identify new methods of use, and to determine the efficacy of treatments with HDIs alone or in combination with other molecules.

So far, molecular studies have identified new HDIs, and combinations of these with other classical agents have been proposed. Since SAHA was approved by the FDA for the treatment of cutaneous T-cell lymphoma in 2006, the clinical use of HDIs in patients with leukemia will be a reality in a short time. Further clinical trials involving a number of HDIs, used either alone or in combination with other antileukemia agents, are needed to consolidate the clinical use of these epigenetics modulators.

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Conflict of Interest

The authors hereby declare that there are no conflicts of interest that might be perceived to influence the discussion reported herein.

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Figure Legends

Table 1. Classical drugs currently in use to treat leukemia and their mechanism of action. Drug class is listed in italics, followed by the medication name in bold.

Table 2. Main histone deacetylase inhibitors evaluated as adjuvants for the treatment of leukemia.