Drugs of the Future 2011, 36(8): 567-576

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.8.1641634



MONOGRAPH

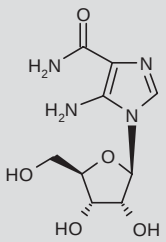
# ACADESINE

Rec INN; BAN; USAN

*5'-AMP-Activated Protein Kinase Inhibitor*
*Apoptosis Inducer*
*Oncolytic*

AICA-riboside
AICAR
ATH-001
Acadra®

5-Amino-1-(β-D-ribofuranosyl)-1*H*-imidazole-4-carboxamide
InChI: 1S/C9H14N4O5/c10-7-4(8(11)17)12-2-13(7)9-6(16)5(15)3(1-14)18-9/h2-3,5-6,9,14-16H,1,10H2,(H2,11,17)/t3-,5-,6-,9-/m1/s1



$C_9H_{14}F_7N_4O_5$
Mol wt: 258.2313
CAS: 2627-69-2
EN: 174642


## SUMMARY

*Acadesine (AICA-riboside, Acadra®) is a well-known small molecule produced by either chemical synthesis or bacterial fermentation. It has undergone extensive clinical development for cardiovascular disorders but never reached the market for these indications due to efficacy concerns. The drug has shown anticancer activity in a wide panel of in vitro and in vivo models of B-cell hematological malignances, without affecting T cells. In these indications, acadesine has a novel mechanism of action mediated by nucleotide pool disruption and which is independent of p53 status, making it effective in B cells resistant to p53-dependent therapies. Acadesine was recently shown to have a predictable and good safety profile at doses associated with antitumor activity in a phase IIa clinical study in chronic lymphocytic leukemia (CLL) patients. Acadesine is undergoing phase II development for the treatment of several B-cell hematological malignances, including CLL, multiple myeloma, mantle cell lymphoma and acute lymphoblastic leukemia.*


C. Campàs-Moya. Advancell, Baldiri Reixac 10-12, 08028 Barcleona, Spain. E-mail: claracampas@gmail.com.
*Synthesis prepared by S. ShankharaRaman, C. Estivill, R. Castañer. Thomson Reuters, Provença 398, 08025 Barcelona, Spain.

## SYNTHESIS*

Acadesine has been prepared by the following routes:

Acylation of inosine (I) with $Ac_2O$ in the presence of pyridine yields inosine triacetate (II), which is then protected as the N-MEM derivative (III) using chloromethyl methyl ether and DIEA in $CH_2Cl_2$. Deacetylation of compound (III) with $NH_3$ in MeOH affords 1-MEM-inosine (IV), which finally undergoes purine ring opening in refluxing NaOH (1). Scheme 1.

In a related method, acadesine is obtained by MEM protection of inosine acetate (II) with chloromethyl methyl ether by means of NaH in dioxane, and subsequent purine ring hydrolysis with NaOH in refluxing EtOH (2). Scheme 1.

Similarly, direct hydrolysis of unprotected inosine (I) with KOH in water at 130 °C produces a mixture of acadesine and 9-β-D-ribopyranosylhypoxanthine, from which acadesine can be isolated using column chromatography (3). Scheme 1.

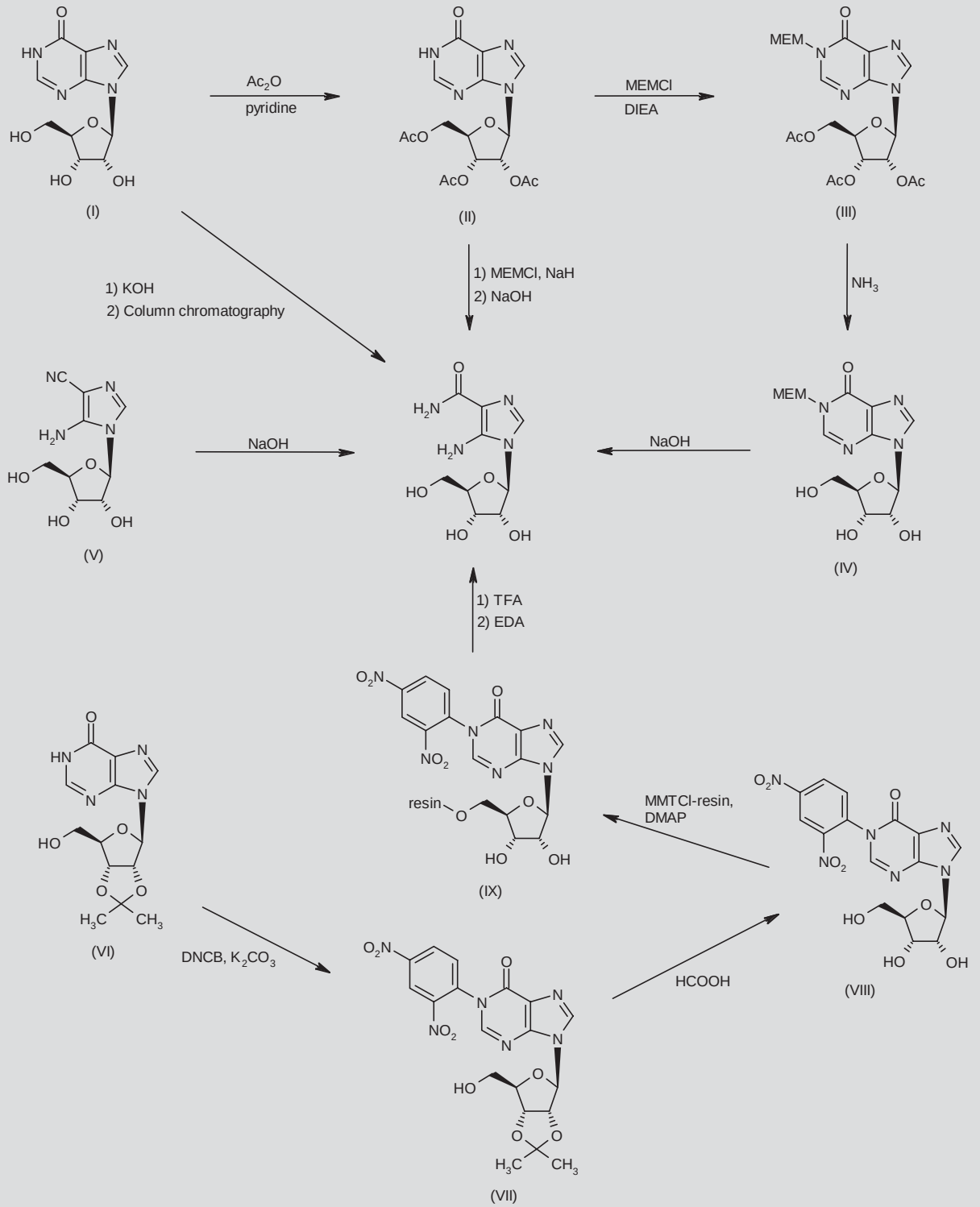
An alternative strategy is the hydrolysis of the imidazolecarbonitrile (V) with NaOH at 100 °C (4). Scheme 1.

In a different approach, reaction of 2',3'-*O*-isopropylideneinosine (VI) with 2,4-dinitrochlorobenzene (DNCB) by means of $K_2CO_3$ at 80 °C yields $N^1$-(2,4-dinitrophenyl)-2',3'-*O*-isopropylideneinosine (VII), which is then *O*-deprotected with HCOOH in $H_2O$ to give 1-(2,4-dinitrophenyl)inosine (VIII). Regioselective attachment of the triol (VIII) to MMTCl resin in the presence of DMAP in pyridine provides the resin-bound compound (IX), which is then submitted to purine ring hydrolysis with EDA in DMF, and subsequent deprotection of the obtained intermediate with TFA in $CH_2Cl_2$ (5). Scheme 1.

The imidazolecarbonitrile precursor (V) is prepared as follows:

Condensation of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide (X) with diaminomaleonitrile (XI) yields the ribofuranosyl diamine (XII), which is alternatively prepared by *N*-protection of diamine (XI) with TMSCl in the presence of HMDS in refluxing acetonitrile and subse-

**Scheme 1.** Synthesis of Acadesine



quent coupling of the obtained bis(silylamine) (XIII) with the D-ribofuranosyl acetate (XIV) by means of TMSOTf in $CH_2Cl_2$. Cyclization of diamine (XII) with $HC(OEt)_3$ in the presence of NaOMe in anisole affords the 4,5-dicyanoimidazole derivative (XV), which undergoes regioselective addition of NaOMe in MeOH to give imidate (XVI). Finally, Hofmann rearrangement of imidate (XVI) with NaOCl in the presence of NaOH yields imidazolecarbonitrile (V) (4). Scheme 2.

Alternatively, formylation of diaminomaleonitrile (XI) with HCOOH in benzene leads to *N*-formyldiaminomaleonitrile (XVII) (6), which is then silylated with TMSCl in the presence of HMDS to yield the protected amine (XVIII). Finally, amine (XVIII) is condensed with the ribofuranosyl acetate (XIV) in the presence of TMSOTf in $CH_2Cl_2$ to give compound (XIX), which undergoes cyclization using AcOH in refluxing benzene yielding nitrile (XV) (4). Scheme 2.

Synthons (X) and (XIV) are synthesized as follows:

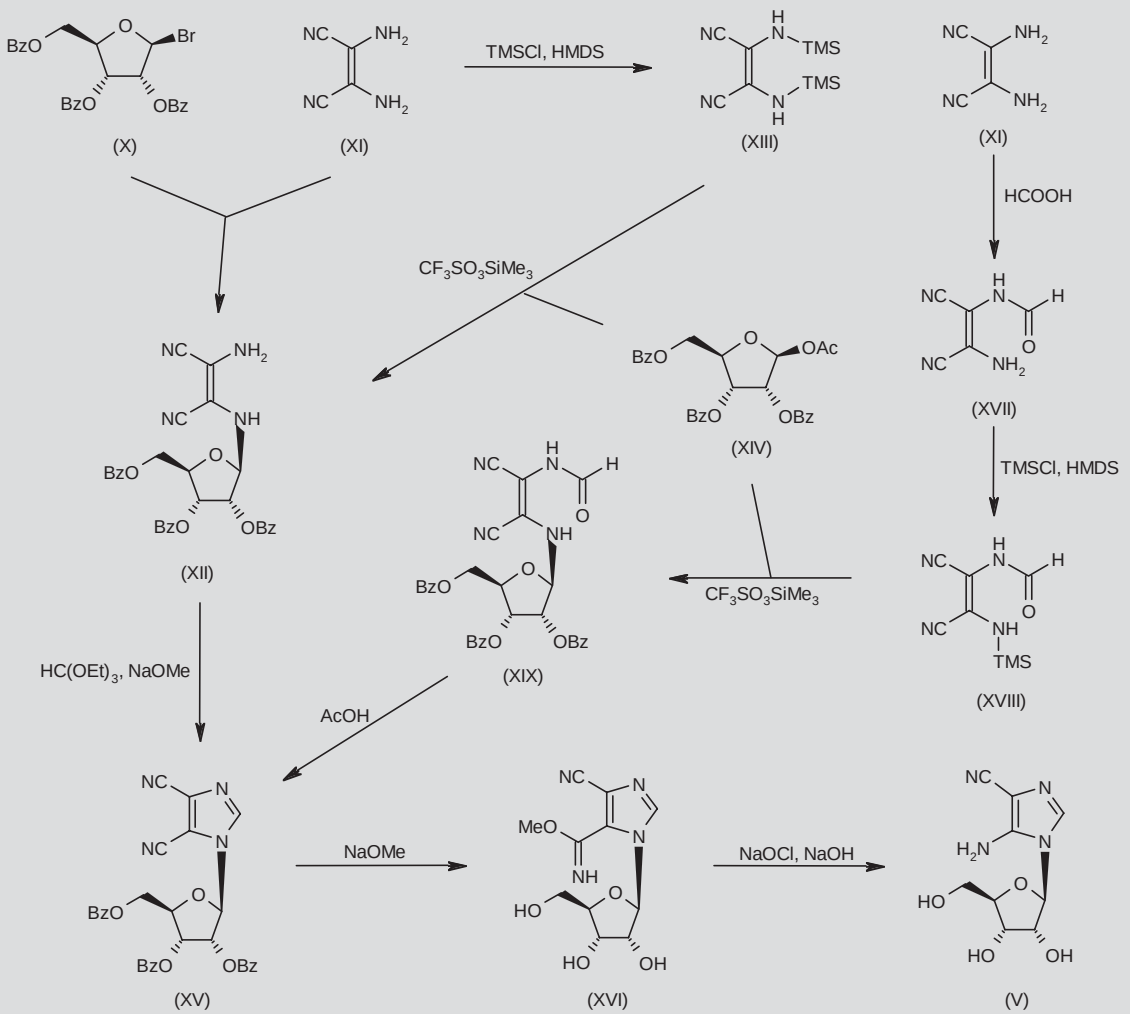
Methylation of D-ribose (XX) with MeOH using HCl yields methyl D-ribofuranoside (XXI), which is then benzoylated with BzCl in $CHCl_3$/pyr to give the protected intermediate (XXII). Bromination of methyl ether (XXII) with HBr in AcOH furnishes bromide (X). Acetylation of methyl ether (XXII) with $Ac_2O$ and AcOH produces acetate (XIV) (7). Scheme 3.

## BACKGROUND

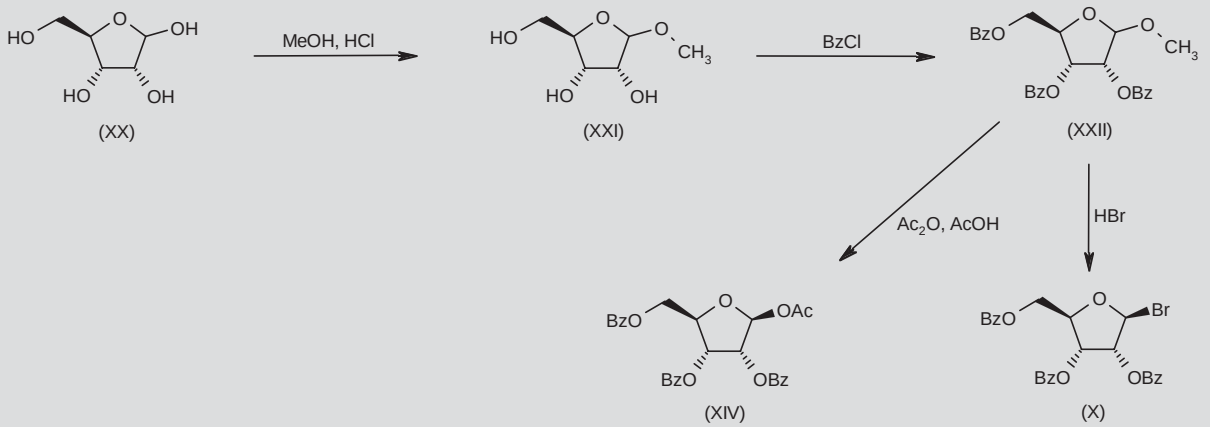
B-cell hematological malignancies comprise a series of leukemia and lymphomas of B lymphocyte origin. Among these cancers are severe conditions, such as chronic lymphocytic leukemia (CLL), multiple myeloma (MM), mantle cell lymphoma (MCL) and acute lymphoblastic leukemia (ALL).

High response rates have been recorded in CLL after combined immunochemotherapy (ICT), which comprises an anti-CD20 monoclonal antibody (rituximab), a nucleoside analogue (fludarabine) and an alkylator (cyclophosphamide). Despite success, these approaches have limitations, with not all patients being eligible for aggressive ICTs. Indeed, fludarabine and cyclophosphamide are

**Scheme 2.** Synthesis of Imidazolecarbonitrile Precursor (V)



**Scheme 3.** Symthesis of Synthons (X) and (XIV)




genotoxic compounds whose effects are not limited to the leukemic population, and serious complications, such as immunosuppression and myelosuppression, can occur. Relapse thus remains universal in CLL, and refractoriness eventually ensues. Survival of patients who become refractory to fludarabine-based approaches is limited because their immunity is very low at this stage and their refractoriness extends to a wide range of potential treatments. One of the main causes of refractoriness is alteration of p53 protein by either gene mutations or chromosomal deletion (deletion in 17p) (8-10).

Although MCL typically responds to frontline chemotherapy, it remains incurable with standard approaches. Recent improvement has been achieved by the successful introduction of monoclonal antibodies and dose-intensified approaches, including autologous stem cell transplant (ASCT) strategies. However, with the exception of allogeneic hematopoietic stem cell transplant (HSCT), current treatment approaches are non-curative and relapse is related to shorter survival, particularly in patients with genetic alterations, such as *TP53* mutations (11). Proteasome inhibitors (bortezomib), serine/threonine-protein kinase mTOR inhibitors (temsirolimus) and immunomodulating drugs (lenalidomide) have recently been added to the treatment options in MCL. Given its unique biology, relative rarity and the difficulty in achieving long-lasting remissions with conventional approaches, patients with MCL are encouraged to participate in clinical trials (12).

For decades, the mainstay of MM therapy have been alkylators and corticosteroids, specifically the oral regimen of melphalan and prednisone, with a median overall survival of approximately 3 years until the late 1990s. Since then, dramatic outcome improvements have occurred owing to the new active agents thalidomide, bortezomib and lenalidomide, ASCT and improvements in supportive care, and the current median survival is in excess of 5 years. The role of ASCT, endpoints of therapy and value of risk stratification are all important areas of debate and disagreement (13). Response to therapy, and thus survival, depends on age, comorbidities and the presence of cytogenetic abnormalities (14). Despite advances in high-dose chemotherapy, stem cell transplant and the development of novel therapeutics, relapse of underlying disease remains the primary cause of treatment failure, particularly in patients with 17p deletions or *TP53* mutations (15, 16).

With current therapies, the vast majority of children with ALL are now long-term survivors. Although ALL is curable in one-third of adult patients (17), results vary greatly on account of different clinical, immunological and cytogenetic/genetic characteristics. These data, along with the kinetics of response to early treatment, help establish the individual risk class with considerable accuracy, and support risk-specific treatments that should warrant optimal results with as little as possible non-relapse mortality. Modern first-line therapy consists of standard- and high-dose chemotherapy, HSCT and new targeted therapies, all integrated with the analysis of prognostic factors and the study of subclinical residual disease for key therapeutic decisions. These changes are improving long-term outcome, with some studies reporting cure rates of up to 50% when using pediatric-inspired therapies in adults (17, 18). Despite significant progress and success in the treatment of ALL, a significant number of patients continue to relapse, and for them, outcome remains poor (17-19).

Acadesine is a small molecule with activity against leukemia and lymphomas of B cell origin through a novel mechanism of action which is independent of cytogenetic alterations, such as *TP53* deletions and mutations. Phase IIa clinical studies have shown that acadesine is safe at doses that demonstrate anticancer activity in CLL patients resistant to other therapies. The favorable efficacy/safety profile of the drug together with its synergistic effects with current therapies make it an optimal candidate for the treatment of B-cell malignancies. Acadesine is under development for the treatment of CLL, MCL, MM and ALL.

## PRECLINICAL PHARMACOLOGY

Acadesine enters cells through specific *S*-(4-nitrobenzyl)-6-thioinosine (NBTI)-dependent transporters (20). After entering cells, it is phosphorylated by adenosine kinase to the corresponding nucleotide, ZMP or AICA-ribotide (AICAR). ZMP is the penultimate intermediate of the de novo synthesis of purine nucleotides and it is further metabolized to inosine monophosphate (IMP), adenosine triphosphate (ATP) and guanosine triphosphate (GTP) and their deoxy counterparts, and from there to their terminal catabolite, uric acid. Depending on the activity of adenosine kinase, which varies according to cell type, the administration of acadesine will result in elevation of the concentration of ZMP and of its more distal products, most notably ATP and uric acid (21).

ZMP has been described to activate 5'-AMP-activated protein kinase (AMPK) by allosteric stimulation due its structural analogy with AMP and by promoting its phosphorylation by upstream kinases. Moreover, it has been reported that AMPK is activated upon change in the AMP:ATP ratio (22).

Acadesine has shown in vitro and in vivo antileukemic activity in a wide panel of non-clinical models of B-cell malignancies, with a selective elimination of B lymphocytes.

In cultured CLL primary cells, acadesine induced apoptosis in a concentration-dependent manner (n = 5), with a half-maximal effective concentration ($EC_{50}$) for apoptosis of 380 ± 60 μM (20). T cells from CLL patients were resistant to acadesine-induced apoptosis at concentrations up to 2 mM. Higher concentrations of acadesine (2-4 mM) only slightly affected the viability of T cells. These results indicated that B cells are much more sensitive than T cells to acadesine-induced apoptosis. ZMP accumulation induced by acadesine in CLL cells has been confirmed by HPLC analysis, showing that intracellular levels of ZMP in B cells are three times higher than in T cells, indicating that lack of accumulation may be the reason why acadesine does not induce cell death in T lymphocytes. The study also showed that B cells cultured from CLL patients resistant (in vivo and ex vivo) to fludarabine were as sensitive to acadesine as B cells from fludarabine responders.

In CLL cells, acadesine induces both ZMP accumulation and ATP depletion, and AMPK is thus activated and phosphorylated (20). However, AMPK appears not to be involved in acadesine-induced apoptosis, since more specific AMPK activators such as phenformin or A-769662 do not promote cell death in CLL cells and acadesine-mediated cell death can occur efficiently in the absence of AMPK (23). In accordance with this, acadesine also decreases intracellular ATP levels through an AMPK-independent mechanism in hepatocytes (24).

Mutational status of immunoglobulins (IgVH) and tyrosine-protein kinase ZAP-70 expression are two main prognostic factors for CLL. It has been described that the unmutated version of IgVH leads to a more aggressive disease with shorter survival time (25). The cytogenetic profile also determines response to treatment and overall survival in CLL (8). A study conducted in primary CLL cells showed that sensitivity to acadesine was independent of IgVH mutational status (28 unmutated cases and 72 mutated cases were assessed). Also, no correlation was found between ZAP-70-positive (n = 38) and ZAP-70-negative cases (n = 63) at any of the concentrations tested (26).

The effect of acadesine on other mature B-cell neoplasms, such as follicular lymphoma (FL), MCL, Waldenström's macroglobulinemia (WM) and splenic marginal zone B-cell lymphoma (SMZL), were investigated in primary cells cultured ex vivo (27). A concentration-dependent decrease in B cell viability in the 21 SMZL samples analyzed was observed. Acadesine induced apoptosis in B cells from seven of the eight MCL patients and from one sample of FL. B cells from one MCL patient and four of the five FL samples were resistant to acadesine at concentrations up to 2 mM. At the concentration of 2 mM, acadesine did not induce apoptosis in T cells from any of the samples tested, which was consistent with the results with CLL samples discussed above (20). These data further demonstrated the high selectivity of acadesine towards B cells. The $EC_{50}$ observed in MCL primary cells and SMZL primary cells was slightly higher than the $EC_{50}$ observed in CLL cells. However, an increased number of samples should be analyzed to further confirm this (20, 27).

The antineoplastic activity of acadesine was tested in vivo in a mouse MCL xenograft model, showing a significant reduction in tumor growth either alone or in combination with other agents, such as the anti-CD20 antibody rituximab. Severe combined immunodeficiency (SCID) mice were inoculated s.c. with $10^7$ human B-lymphoma JeKo-1 cells. At day 12 post-inoculation, mice were randomized and administered either 400 mg/kg acadesine 5 days/week, rituximab 10 mg/kg/week, a combination of both drugs or vehicle for 18 days. Administration of acadesine significantly reduced tumor burden when compared to control animals, as soon as 7 days of treatment. Acadesine induced a 50% reduction in tumor size, similar to the reduction seen with rituximab. Importantly, a potent synergistic effect was observed with the combination of acadesine and rituximab, with a reduction of tumor size to 95% ($P < 0.01$) (28).

Acadesine induced apoptosis and cell cycle arrest in a panel of MM cell lines bearing different mutations and cytogenetic alterations, and thus representing different MM variants. In this study, Baumann et al. showed that acadesine inhibited cell growth in MM cell lines (NCI-H929, U266, RPMI 8226 and OPM-2) carrying different cytogenetic alterations. Acadesine also induced cell cycle arrest in the four cell lines tested and led to phosphorylation and activation of AMPK. These effects required the uptake of acadesine into the cells and its phosphorylation to ZMP. Acadesine treatment led to the inhibition of several pathways, such as basal extracellular signal-regulated kinase (ERK), mammalian target of rapamycin (mTOR) and P70S6 kinase (P70S6K), as well as Akt phosphorylation, and blocked IL-6, insulin-like growth factor I (IGF-I) and human stromal cell HS-5-conditioned medium-induced increase of cell growth (29).

The antineoplastic activity of acadesine was further investigated in vivo in a mouse MM xenograft model. Acadesine showed a significant reduction in tumor growth either alone or in combination with other agents, such as bortezomib and lenalidomide (30). To that purpose, SCID mice were inoculated s.c. with MM1S cells. When the tumor reached a volume of 100 $mm^3$, mice were randomized to receive vehicle, acadesine 200 mg/kg, bortezomib 0.5 mg/kg, dexamethasone 0.5 mg/kg or a combination of these drugs for 33 days. Acadesine 200 mg/kg and bortezomib 0.5 mg/kg were administered i.p. daily, whereas dexamethasone 0.5 mg/kg was administered i.p. 2 days a week. Both acadesine and bortezomib monotherapy induced a reduction in tumor size, while dexamethasone alone

did not induce any decrease in tumor size. In this study, bortezomib alone was the most effective single-agent treatment. Importantly, the combination of acadesine and bortezomib was more effective than either of the drugs alone. The most striking results were those obtained with the triple combination of acadesine, dexamethasone and bortezomib, which induced practically complete tumor remission. Similar results were obtained with acadesine in combination with lenalidomide.

The effect of acadesine has also been investigated in preclinical ALL models. Treatment with acadesine inhibited cell proliferation, induced cell cycle arrest in the $G_1$ phase and induced apoptosis in several childhood ALL cell lines with either chemotherapy-sensitive or -resistant phenotypes. These effects required the uptake of acadesine into the cells and its phosphorylation to ZMP (31). In a study by Leclerc et al., the mechanism of action of acadesine in ALL cells was further explored. The authors described that acadesine treatment induced AMPK phosphorylation, and an upregulation of phospho-Akt and decrease of phospho-mTOR expression and downstream signaling. Akt activation was mediated by AMPK-induced IGF-I receptor activation via phosphorylation of the insulin receptor substrate 1 (IRS-1). Combined therapy simultaneously targeting IGF-I receptor, AMPK, Akt and mTOR pathways resulted in synergistic growth inhibition and cell death (32).

Tumor suppressor p53 defects are rare among patients with newly diagnosed CLL, but are more prevalent (30%) among patients with advanced and chemotherapy-resistant disease (33). In keeping with the fact that p53-mediated apoptosis underpins the cytotoxicity of many anticancer drugs, p53 defects in CLL have been strongly linked to resistance to alkylating agents and purine analogues in ex vivo experiments, in retrospective clinical studies and, most importantly, in three large prospective clinical trials of first-line chemotherapy. In the German CLL4 study comparing fludarabine with fludarabine–cyclophosphamide (34), p53 deletion was found to be the most powerful predictor of therapeutic resistance and short progression-free survival, and the only predictor of short survival in a multivariate analysis (35). To study the role of p53 in acadesine-induced apoptosis, its effects on p53 protein levels and p53 phosphorylation were investigated. Drugs that induce apoptosis with a mechanism dependent on p53 induce phosphorylation of the protein, which leads to its stabilization and accumulation. When CLL cells were incubated with 0.5 mM acadesine, there was no effect on p53 accumulation or p53 phosphorylation, indicating that acadesine-induced apoptosis is p53-independent (20). Moreover, deletions in 17p did not affect response to acadesine in primary CLL cells (23, 26, 36). This is an important difference with respect to other nucleoside analogues, including fludarabine, which require p53 activation for their cytotoxic effect (36).

## PHARMACOKINETICS AND METABOLISM

The pharmacokinetics and metabolism of acadesine have been extensively reported in both preclinical and clinical studies. In mammals, acadesine is primarily taken up and metabolized by erythrocytes, fibroblasts and cardiac and skeletal muscle (37) through the de novo synthesis of purines.

After i.v. administration of radiolabeled [2-$^{14}$C]-acadesine to rats, the major metabolite present in blood was allantoin, an end product of purine metabolism, and ZMP, which was only found in erythrocytes (38). The drug was eliminated from blood with a $t_{1/2}$ of 15 minutes. Almost 60% of the i.v. dose was excreted in the urine and only 2% in the feces. Allantoin (a product of purine metabolism) was the major (85%) metabolite present in the urine, together with small (< 10%) amounts of intact acadesine, indicating extensive metabolism of the drug. Following oral administration of [2-$^{14}$C]-acadesine to rats, about 45% of the dose was excreted in the urine as allantoin, whereas the feces contained 26% of the radioactivity as a mixture of allantoin, xanthine, hypoxanthine and other unidentified products that were probably the result of the metabolism of unabsorbed acadesine by the intestinal microflora.

The pharmacokinetics of acadesine were investigated in healthy male subjects in two separate studies (39, 40). The first trial was a placebo-controlled, double-blind study in 24 healthy men (39). The safety and kinetics of the drug were evaluated after oral and i.v. administration of 10, 25, 50 and 100 mg/kg acadesine. At each dose level, four subjects received active drug and two subjects received placebo. Each volunteer received a dose by 30-minute i.v. infusion and, after a 1-week washout, the same dose by oral administration. Acadesine post-infusion plasma concentrations declined rapidly in a biphasic fashion, and the terminal elimination phase had a harmonic mean $t_{1/2\beta}$ of 1.4 hours. Total plasma clearance, mean residence time and volume of distribution at steady state were 2.5 L/h/kg, 0.7 hours and 1.6 L/kg, respectively. Acadesine was not protein-bound and there was rapid uptake and phosphorylation in red blood cells (RBCs) to its 5′-monophosphate nucleotide ZMP. Renal clearance was 0.2 L/kg/h, with only 8% of the i.v. dose excreted in the urine as intact acadesine. There were no significant differences in the mean estimates of the pharmacokinetic parameters associated with dose, but there was a trend towards a decrease in renal clearance with increasing dose ($P > 0.05$). After oral application, the bioavailability of acadesine was below 5%.

The objective of the second trial (40) was to investigate the disposition and metabolism of acadesine after a 15-minute i.v. infusion of 25 mg/kg of [2-$^{14}$C]-acadesine in four healthy male volunteers. Intact acadesine was only measurable for 2 hours after infusion. The post-infusion total $^{14}$C concentrations declined in a multiexponential manner and the terminal phase had an apparent $t_{1/2}$ of about 1 week. Total plasma clearance was 2.2 ± 0.2 L/h/kg, the acadesine blood/plasma ratio was unity and plasma protein binding was negligible (about 1%). Uric acid, the end product of purine metabolism in humans, was the major metabolite of acadesine in plasma and accounted for all of the total plasma $^{14}$C at 6 hours after infusion. In whole blood, acadesine 5′-monophosphate was present in RBCs, and the nucleotide represented 30% of the total blood $^{14}$C at the end of the infusion. The nucleotide was confined to the RBCs and was not present in plasma. Urine and fecal recoveries over 2 weeks accounted for 48% of the total $^{14}$C dose, with 44% excreted in urine and 4% in the feces. Only 5% of the dose was excreted in urine as intact acadesine. Uric acid was the major metabolite in urine, together with small amounts of hypoxanthine. There was no evidence of conjugation of acadesine or its metabolites with glucuronic acid. Thus, it is concluded that acadesine is metabolized to uric acid through normal purine pathways. Acadesine metabolites also enter the endogenous purine pools and are distributed throughout the body.

A multicenter, dose-escalation phase I/IIa study designed to assess the safety, efficacy and pharmacokinetics of acadesine was conducted. Twenty-four CLL patients received increasing doses of acadesine ranging from 50 mg/kg to 315 mg/kg as a 4-hour i.v. infusion. Preliminary pharmacokinetic results showed that saturation of the metabolism of acadesine to ZMP occurred at 210 mg/kg (41, 42).

## SAFETY

In the early nineties, three pharmacological applications were identified for acadesine (24): 1) stimulation under ischemic conditions of the cardiac production of the vasodilator adenosine, therefore of interest during coronary artery bypass graft (CABG) surgery; 2) inhibition of hepatic gluconeogenesis at the level of fructose-1,6-bisphosphatase, of therapeutic potential in diabetes; and 3) stimulation of AMPK, initially applied to inhibit the hepatic synthesis of triglycerides and cholesterol. Although the drug has not been marketed for these indications due to lack of statistically significant efficacy, the clinical studies performed with acadesine in these indications provide a large safety dataset of over 3,000 patients treated with the agent.

The first trial describing the clinical safety of acadesine was a placebo-controlled, double-blind study performed in 24 healthy men. The safety and kinetics of the drug were evaluated after oral and i.v. administration of 10, 25, 50 and 100 mg/kg. Acadesine was well tolerated in the 24 men treated by both the i.v. and oral routes. No serious adverse events were reported and no clinically significant changes in vital signs were observed. No local i.v. site toxicity in subjects who were treated with the drug was observed (39).

The effect of acadesine on exercise-induced myocardial ischemia was studied in patients with chronic stable angina pectoris. The safety profile of the drug in terms of vital signs, biochemical and hematological variables, and adverse events, was also assessed. Twelve patients were entered into a 5-way, randomized, double-blind study comparing the effects of 4 doses of acadesine (6, 12, 24 and 48 mg/kg as a 50-minute i.v. infusion) with placebo, administered at approximately weekly intervals. Acadesine infusion had no significant effect on the full blood count or prothrombin time. Dose-related asymptomatic and transient hyperuricemia occurred on five occasions during drug infusion at the higher doses. Asymptomatic hypoglycemia occurred in two patients with 48 mg/kg acadesine. There were no alterations in biochemical markers or renal or hepatic function (43).

In 1994, the results of a multicenter study that evaluated the safety and the effects of acadesine on myocardial ischemia and left ventricular function in patients undergoing coronary artery bypass graft (CABG) surgery were reported. The study enrolled 116 patients who were randomized to receive placebo, low-dose (0.05 mg/kg/min) or high-dose (0.1 mg/kg/min) acadesine as a continuous i.v. infusion for 7 hours. The study concluded that the administration of i.v. acadesine for 7 hours was safe in patients undergoing CABG surgery. Thirty-four of these patients were further analyzed in 1995 to determine acadesine-induced changes in granulocyte CD11b expression, and no adverse effects for acadesine were reported (44, 45).

In a study conducted to determine the efficacy of acadesine in restoring post-ischemic function in patients undergoing CABG, 100 patients were randomized to receive either 0.1 mg/kg/min acadesine as an i.v. infusion over 7 hours or placebo. No adverse effects were reported in this study (46).

No adverse events were reported in a study conducted in 8 healthy men treated with acadesine 100 mg/kg by i.v. infusion over 90 minutes to evaluate its effects on platelet aggregation ex vivo (47).

In 1995, Page et al. investigated the efficacy of acadesine in the treatment of Lesch-Nyhan syndrome. One patient received i.v. acadesine infusions on 12 consecutive days with varying doses of 150-1,300 mg/h. The exact duration of infusions was not defined in the report, although the respective figure indicated infusion durations up to more than 6 hours. Acadesine treatment was accompanied by concomitant administration of 400 mg/day allopurinol. Significantly, no acadesine was detected in cerebrospinal fluid before or after therapy. The authors concluded that a larger dose administered with longer-lasting infusions could have been employed safely (48).

In 20 medical centers in the U.S., 633 patients undergoing CABG were randomized in a blind fashion to receive either placebo (n = 212), low-dose acadesine (0.05 mg/kg/min; n = 214) or high-dose acadesine (0.1 mg/kg/min; n = 207) by 7-hour i.v. infusion. The aim of the study was to determine the efficacy of acadesine in preventing myocardial infarction and adverse cardiac outcomes after CABG. No adverse effects due to acadesine were reported in this study (49). In a similar study, the effect of acadesine on the incidence of myocardial infarction, adverse cardiovascular outcomes and mortality was assessed in 821 patients undergoing CABG. They were randomized in a double-blind manner to placebo (n = 418) or acadesine (n = 403) by i.v. infusion over 7 hours (0.1 mg/kg/min) and via the cardioplegic solution (5 μg/mL). No adverse events were related to acadesine treatment. Hemodynamic measurements, including systolic and diastolic blood pressure and heart rate taken during the perioperative period, did not reveal any significant differences between the treatment groups. With the exception of mild, transient elevation of serum uric acid levels at the end of the acadesine infusion (27% above baseline level), there were no differences between the placebo and the acadesine group. Elevations in uric acid were transient and without clinical sequelae (50).

Individual patient data from 5 randomized, placebo-controlled, double-blind clinical trials including 81 international medical centers in the U.S., Canada and Europe were reported in 1996. These clinical trials included a total of 4,043 patients undergoing CABG surgery and randomized to receive either placebo (n = 2,031) or 0.1 mg/kg/min acadesine (n = 2,012) by i.v. infusion for 7 continuous hours and via the cardioplegic solution. Regarding safety, the study concluded that the incidence of adverse events was similar in the acadesine versus the placebo groups in all the individual studies and across all the studies. Acadesine did not affect blood pressure or cardiac electrical conduction. A transient increase in serum uric acid in the acadesine group was observed, but resolved during hospital stay without clinical sequelae (51). A report related to the previous studies was published in 2006. The purpose of this report was to assess the safety and efficacy of acadesine for reducing long-term mortality among patients with post-reperfusion myocardial infarction. At 54 institutions, 2,698 patients undergoing CABG surgery were randomized to receive placebo (n = 1,346) or acadesine (n = 1,352) by i.v. infu-

sion (0.1 mg/kg/min for 7 hours). The study concluded that acadesine was safe and useful for reducing long-term mortality among patients suffering post-reperfusion, perioperative myocardial infarction. No long-term safety concerns were reported (52).

A study was conducted in the U.K. to determine whether acadesine stimulates muscle glucose uptake in humans. Acadesine was administered to 10 healthy volunteers as a single i.v. dose at a rate of 10 mg/kg/h over a maximum period of 3 hours (maximum dose given was 30 mg/kg). No information was provided regarding any adverse events reported. From the results obtained, it was concluded that the acadesine infusion caused a twofold increase in skeletal muscle glucose uptake after 3 hours in healthy young male volunteers (53). Ten male patients with type 2 diabetes (mean age: 64 ± 2 years) were treated with a single i.v. dose of 90 mg/kg acadesine as a 2-hour infusion to assess the impact of acadesine on glucose and fatty acid metabolism in vivo. Although safety was not an endpoint for this study, no safety concerns were reported (54).

A multicenter, dose-escalation phase IIa study designed to assess the safety, efficacy and pharmacokinetics of acadesine in CLL patients with refractory or relapsed disease was recently reported. The results of this study demonstrated that acadesine has an acceptable safety and tolerability profile at doses that induce a reduction in the leukemic tumor burden. The study population included patients with relapsed or refractory CLL who had received a minimum of one prior line of treatment, including either a fludarabine- or an alkylator-based regimen (median number of prior treatments was five). The study was conducted in 2 parts and enrolled 24 patients in cohorts of 3 patients each. In part I, acadesine was administered as a single dose with the aim of finding the optimal biological dose (OBD), defined as the dose of acadesine that generated the maximum exposure to the active metabolite ZMP with no dose-limiting toxicity (DLT). Acadesine 315 mg/kg was the DLT dose, with one patient having a DLT of tumor lysis syndrome that resolved with appropriate treatment. Saturation of the transformation of acadesine to ZMP occurred at 210 mg/kg. Thus, the OBD was declared to be 210 mg/kg and this was the dose used for multiple acadesine administrations in part II of the study. In part II, patients received up to five doses of acadesine. Reversible asymptomatic hyperuricemia was observed in some patients in part I and was significantly reduced in incidence with the introduction of mandatory prophylactic allopurinol in subsequent cohorts. Importantly, acadesine did not induce myelosuppression at any of the doses tested. No grade 3 or 4 adverse events occurred at the OBD and no grade 5 events occurred in the study (41, 42).

## CLINICAL STUDIES

An open-label, dose-escalation phase II study has been conducted by Advancell (41, 42). The primary objective of the study was to demonstrate the safety and tolerability of acadesine in patients with CLL. Secondary objectives were to determine the pharmacokinetics of acadesine and its metabolite ZMP, and to determine the OBD of acadesine in patients with CLL. Conventional efficacy as per Cheson's criteria (54) was not measured in this study, but trends for efficacy were evaluated using pharmacodynamic parameters: B-cell and T-cell counts in peripheral blood and lymph node size (only palpable lymph nodes). The patient population was described as CLL patients with refractory or relapsed disease who had received one or more prior lines of treatment, which must have included either a fludarabine- or an alkylator-based regimen. Refractoriness was defined as any patient who failed to achieve a complete or partial response according to the National Cancer Institute-Sponsored Working Group guidelines for CLL. Fludarabine refractoriness also included patients who achieved a complete or partial response of less than 6 months' duration. The study was conducted in two parts. In part I, patients were treated with single doses of acadesine and part II tested multiple doses. Dose escalation was conducted in cohorts of three to six patients each. A data monitoring board (DMB) reviewed the safety, efficacy and pharmacokinetic data after each cohort to recommend the dose and schedule of acadesine in the following cohort. A total of 24 patients were included in the study. All patients had a diagnosis of CLL except for one patient who had a diagnosis of small cell lymphocytic lymphoma. In part I, acadesine was administered as a single dose with the aim of finding the OBD, defined as the dose of acadesine that generated the maximum exposure to the active metabolite ZMP with no DLT. Eighteen patients received acadesine as a single 4-hour infusion at doses of 50 mg/kg (6 patients), 83.5 mg/kg (3 patients), 139.5 mg/kg (3 patients), 210 mg/kg (3 patients) and 315 mg/kg (3 patients). Acadesine 315 mg/kg was the DLT dose, with one patient having a DLT of tumor lysis syndrome that resolved with appropriate treatment. Saturation of the metabolism of acadesine to ZMP occurred at 210 mg/kg. Thus, the OBD was declared as 210 mg/kg and this was the dose used for multiple acadesine administrations in part II of the study.

In part II, patients received up to five doses of acadesine. Cohort I of part II of the study consisted of two consecutive doses at 210 mg/kg (OBD). Three patients were treated in this cohort and a decrease in absolute B cell count was observed ranging from 6% to 35% with respect to the B cell count prior to acadesine administration. Cohort 2 of part II of the study consisted of 5 consecutive doses at 210 mg/kg on days 1, 4, 8, 11 and 15. Also, three patients were treated in this cohort. A decrease in absolute B cell count was observed in two patients, and in the patient in whom no B cell decrease was observed, a reduction of 75% in lymph node mass was reported.

Of note, evidence for antileukemic activity was observed. Overall, the majority of patients treated with acadesine at the OBD presented a decrease in absolute B cell count number, a reduction of clinically palpable lymphadenopathies, or both. One patient with a p53 deletion presented sustained antileukemic activity after a single dose of acadesine. Two patients presenting with symptomatic CLL-related neuropathic pain and skin infiltration had resolution and improvement of these symptoms, respectively. After cohort II of part II, the DMB considered that the main endpoints of the study had been reached and the study was closed.

The favorable efficacy/toxicity profile of acadesine together with its synergistic effects with current therapies makes it an optimal candidate for the treatment of B-cell malignancies. In CLL and MCL, acadesine combinations with ICT could improve the quality of response to increase the rate of eradication of minimal residual disease, as well as be an alternative treatment in patients with a dysfunctional p53 pathway, which implies much-reduced sensitivity to ICT, and in patients who have contraindications to aggressive ICT (21,

41, 42). These approaches will be investigated in phase IIb studies in patients with MCL, CLL and MM.

## SOURCE

Advancell (ES).

## DISCLOSURES

The author is an employee of Advancell.

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