Received: 29 May 2019 | Revised: 25 June 2019 | Accepted: 26 June 2019

DOI: 10.1002/prp2.506

INVITED REVIEW

Therapeutic potential of adenosine kinase inhibition—Revisited

Michael F. Jarvis

Global Medical Affairs, Abbvie, Inc, North Chicago, Illinois

Correspondence
Michael F. Jarvis, Global Medical Affairs, R4MA ABV1-5NE, 1 North Waukegan Rd., North Chicago, IL 60064.
Email: michael.jarvis@abbvie.com

Funding information
None.

Abstract

Adenosine (ADO) is an endogenous protective regulator that restores cellular energy balance in response to tissue trauma. Extracellular ADO has a half-life of the order of seconds thus restricting its actions to tissues and cellular sites where it is released. Adenosine kinase (AK, ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20) is a cytosolic enzyme that is the rate-limiting enzyme controlling extracellular ADO concentrations. Inhibition of AK can effectively increase ADO extracellular concentrations at tissue sites where pathophysiological changes occur. Highly potent and selective nucleoside and non-nucleoside AK inhibitors were discovered in the late 1990s that showed in vivo effects consistent with the augmentation of the actions of endogenous ADO in experimental models of pain, inflammation, and seizure activity. These data supported clinical development of several AK inhibitors for the management of epilepsy and chronic pain. However, early toxicological data demonstrated that nucleoside and non-nucleoside chemotypes produced hemorrhagic microfoci in brain in an apparent ADO receptor-dependent fashion. An initial oral report of these important toxicological findings was presented at an international conference but a detailed description of these data has not appeared in the peer-reviewed literature. In the two decades following the demise of these early AK-based clinical candidates, interest in AK inhibition has renewed based on preclinical data in the areas of renal protection, diabetic retinopathy, cardioprotection, and neurology. This review provides a summary of the pharmacology and toxicology data for several AK inhibitor chemotypes and the resulting translational issues associated with the development of AK inhibitors as viable therapeutic interventions.

KEYWORDS
adenosine, adenosine kinase, analgesia, inflammation, motor activity, seizures

Abbreviations: 5′d-5IT, 5′-deoxy,5-iodotubercidin; 5-IT, 5-iodotubercidin; A-134974, N7-((1′R,2′S,3′R,4′S)-2′,3′-dihydroxy-4′-amino-cyclopentyl)-4-amino-5-iodo-pyrrolo[2,3-a] pyrimidine; A-286501, N7-((1′R,2′S,3′R,4′S)-2′,3′-dihydroxy-4′-amino-cyclopentyl)-4-amino-5-bromo-pyrrolo[2,3-a]pyrimidine; ABT-702, 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3,-d]pyrimidine; ADO, Adenosine; AK, Adenosine Kinase (ATP:adenosine 5′-phosphotransferase); CD39, NTPD (EC 3.6.1.5), ecto nucleoside triphosphate diphosphohydrolase; CD73, (EC 3.1.3.5), ecto-5′-nucleotidase; NH2dADO, 5′amino,5′-deoxyadenosine; NPP, (EC 3.6.1.9, EC 3.1.4.1.) ectonucleotide pyrophosphatase/phosphodiesterase; NT, nucleoside transporter.

1 | INTRODUCTION

It is well established that adenosine (ADO) functions to restore energy balance in cells that have been exposed to stressors or trauma.[1-4] As such, ADO exerts protective effects in a broad spectrum of pathological conditions including inflammation, various forms of neuronal hyperexcitability, and/or toxicity including hypoxia, seizures, and chronic pain.[5,6] These cellular protective actions of ADO support its classification as a "retaliatory" or "homeostatic" modulator of cellular activity.[7]

ADO is only one component of a purinergic signaling cascade as its availability is tightly regulated by multiple enzymes and transporters.[2] As illustrated in Figure 1, the intracellular and extracellular metabolic degradation of ATP to its downstream metabolites adenosine diphosphate (ADP), adenosine monophosphate (AMP), and ADO generates an ensemble of ligands that act at multiple cell surface receptor families termed P1 and P2 receptors.[1,8-10] The P1 receptors are specifically activated by ADO and comprise a family of G protein-coupled receptors (A_1, A_{2A}, A_{2B}, A_3).[2,6] The P2 receptor family comprises subfamilies of G protein-coupled P2Y receptors and ligand-gated P2X receptors.[10]

2 | BIOCHEMISTRY

Under physiological conditions, cellular uptake and metabolic conversion regulates ADO extracellular concentrations. This process is rapid (seconds) and restricts ADO actions to local tissues and cellular sites where it is released.[11,12] Adenosine kinase (AK, ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20) is a cytosolic enzyme that catalyzes the phosphorylation of ADO to AMP and is one of the two enzymes responsible for ADO metabolism. ADO deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4) also contributes to ADO conversion, but AK-mediated metabolism of ADO is the primary regulator under physiologic conditions.[13] The mammalian AK enzyme has been cloned[14,15] and crystallized.[16] Differential splicing and promoter activity results in two mammalian isoforms of AK, termed long and short, that differ based on the length of exon 1.[17] These isoforms are also differentially localized in the intracellular compartment with the long form found in the nucleus and the short form in the cytoplasm.[17] While the physiological significance of the specific isoforms remains to be determined, a role for nuclear AK in transmethylation reactions has been proposed.[17] Functional AK contains two ADO binding sites, a catalytic site with high affinity for ADO and a lower affinity regulatory $MgATP^{2-}$ binding site.[18,19] Extracellular ADO concentrations are governed by an ADO-specific transport system that operates as a non-concentrative, bidirectional, facilitated diffusion transporter. Thus, AK inhibition has the net effect of decreasing cellular reuptake of ADO resulting in an increase in the local concentration of ADO in the extracellular compartment.[20] The molecular biology, enzymology, and biochemistry of AK have been comprehensively reviewed.[17]

3 | PHYSIOLOGY

The dynamic intracellular regulation of ADO by AK supports a mechanistic hypothesis that the intracellular blockade of AK may effectively enhance extracellular ADO concentrations in cells

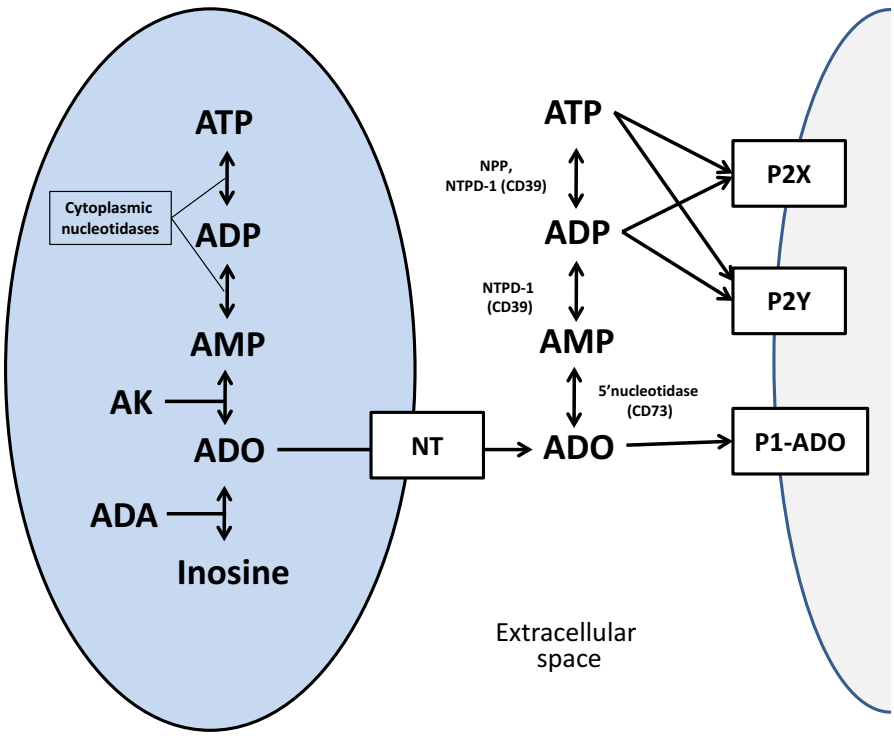

FIGURE 1 The Purinergic Cascade. Intracellular and extracellular concentrations of ATP, ADP, AMP, and ADO are highly regulated by multiple intracellular and extracellular enzymes. The phosphorylation of intracellular ADO to AMP is mediated primarily by the action of AK. The sequential hydrolysis of ATP to ADO via the CD39/CD73 pathway can lead to physiologically relevant extracellular ADO concentrations in certain pathological situations such as inflammation[17,27]

undergoing accelerated ADO release[21] and this process may be more pronounced at tissue sites where pathophysiological changes result in ADO release.[22,23] Proof of this concept has been demonstrated in hippocampal and spinal cord slices in vitro[24,25] and in vivo following peripheral inflammation[26] and during excitotoxic insults to rat striatum in vivo using microdialysis techniques.[23] These latter reports provide compelling evidence that systemically administered AK inhibitors can elicit "a site and event specific" enhancement of endogenous ADO levels.[22,23] Furthermore, AK inhibitors can amplify the actions of ADO independent of a single ADO receptor subtype. This action may be of potential advantage in cases where a multiplicity of ADO receptor subtypes is involved in the protective actions of ADO, such as in inflammation or chronic pain.[2,6]

4 | ADO-BASED DRUG DISCOVERY

The beneficial actions of ADO across a multitude of organ systems coupled with robust effects in experimental models of pathophysiology have served as a basis for rational drug design of ADO-based interventions spanning at least eight decades.[27] ADO-based drug discovery research has encompassed virtually all peripheral and central nervous system diseases and significant pharmaceutical company efforts have been devoted to the potential treatment of cardiovascular and neurodegeneration diseases.[6,8,9,28,29] Despite many years of "drug-hunting" research directed at ADO-mediated interventions, few drugs have advanced into clinical use. Only one drug is currently approved for use in the United States, an A_{2A} receptor agonist for diagnostic cardiac imaging.[30] Additionally, an A_{2A} receptor antagonist has been approved in Japan for the management of Parkinsonian symptoms.[30] A primary limiting factor in the advancement of ADO-based therapeutics is the fact that ADO modulation is highly relevant in essentially all organ systems such that mechanism-based peripheral or central side-effects or tolerability issues are difficult to avoid or modulate.[6,27] Given the ubiquity of homeostatic ADO modulation of physiological systems, the hypothesis regarding the site and event specificity afforded by inhibition of AK[22,23] represented a viable therapeutic approach that offered the potential for improved benefit/risk profile as compared to direct-acting agonists.[28]

Multiple classes of potent and selective nucleoside and non-nucleoside inhibitors (Figure 2) of AK have been generated as a result of rational drug design and optimization of novel screening leads in the late 1990s.[5,31-33] In addition to their potent affinity to inhibit AK, many of these chemotypes offer improved cellular penetration compared to ribose-containing prototypic inhibitors such as 5′-deoxy-5′amino ADO (NH_2d-ADO) (Table 1) and retain nanomolar potency to inhibit AK in intact cells. Representative AK inhibitors of each class (ie, ABT-702 and A-286501) where shown to reversibly and competitively block the ADO recognition site in the enzyme and noncompetitively interact with the $MgATP^{2-}$ site.[34,35] These compounds showed equivalent potency in inhibiting both long and short forms of human AK and AK from multiple mammalian species including mouse, rat, dog, and monkey (Table 2). Crystallography studies have shown that nucleoside

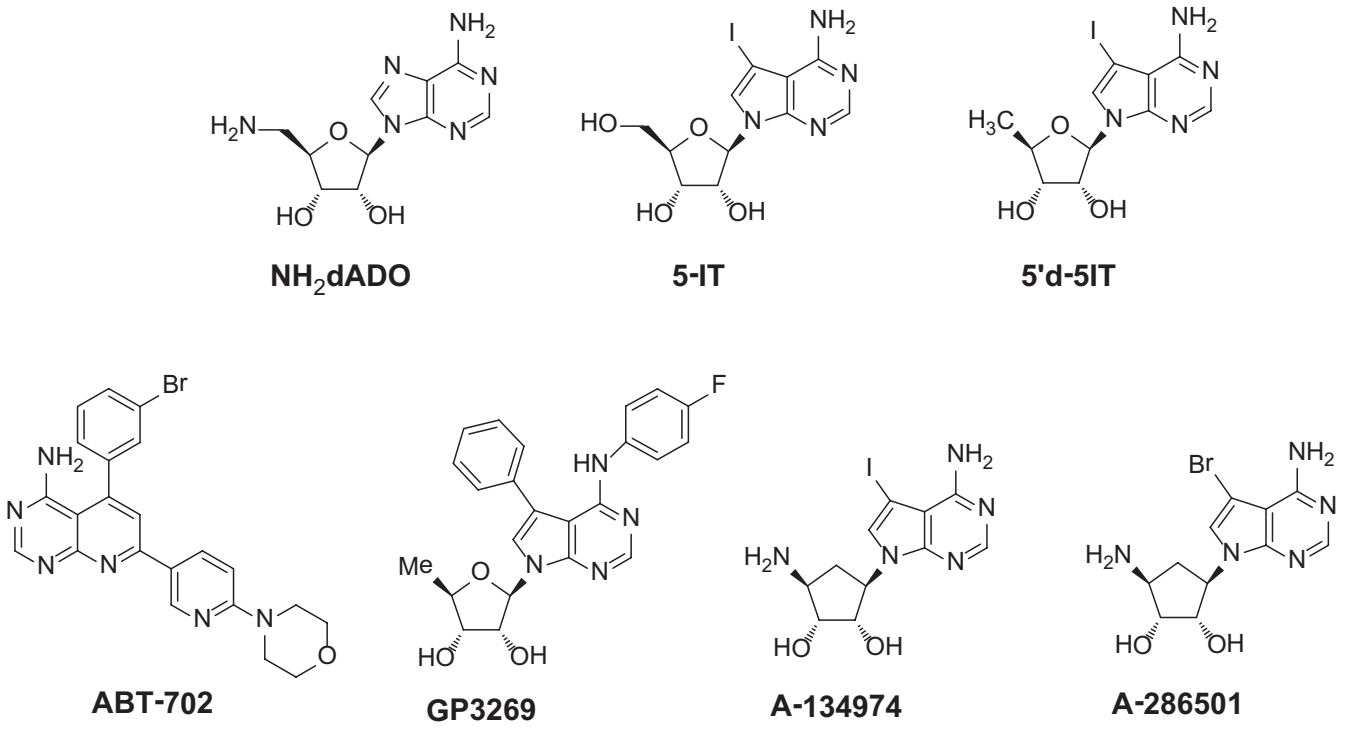

FIGURE 2 Chemical structures of different classes of AK inhibitors

TABLE 1 Pharmacological activity and selectivity of AK inhibitors

IC_{50} nmol/L (±SEM)							
Target	**AK**	**Intact Cell**	**A_1**	**A_{2A}**	**A_3**	**NBTI**	**ADA**
AK inhibitor							
NH_2dADO	15 ± 7	6630 ± 880	>10,000	>10,000	ND	>10,000	ND
5′d-5IT	0.9 ± 0.1	68.1 ± 7.5	>10,000	>10,000	>10,000	>10,000	>10,000
A-134974	0.06 ± 0.07	45 ± 9	>10,000	>10,000	>10,000	>10,000	>10,000
A-286501	0.5 ± 0.1	12 ± 1	>10,000	>10,000	>10,000	>10,000	>10,000
ABT-702	1.7 ± 0.5	51 ± 8	>10,000	2110 ± 1000	>10,000	2220 ± 370	>10,000

Note: Data from [34,35,37,38]. ND, not determined. All data were derived from rat brain except A_3 which is from recombinant human receptors expressed in HEK293 cells.

TABLE 2 K inhibitor potencies across mammalian species

IC_{50} nmol/L (±SEM)			
	5′d-5IT	**A-286501**	**ABT-702**
Human	0.8 ± 0.4	1.4 ± 0.2	3.0 ± 0.8
$hrAK_{long}$	3.0 ± 2.4	1.9 ± 0.1	1.4 ± 0.2
$hrAK_{short}$	1.4 ± 0.3	1.4 ± 0.5	1.2 ± 0.1
Monkey	1.6 ± 0.2	4.6 ± 0.8	1.2 ± 0.4
Dog	2.3 ± 1.0	1.5 ± 0.1	1.3 ± 0.4
Rat	1.4 ± 0.3	0.7 ± 0.1	1.7 ± 0.7
Mouse	1.7 ± 0.7	0.6 ± 0.1	0.8 ± 0.1

Note: Data from [34,35].

and non-nucleoside AK inhibitors bind AK in distinctly different conformations that result in a significant rearrangement of the protein's large and small domains.[36]

Different classes of orally bioavailable and CNS-penetrant AK inhibitors have been shown to be systemically active in diverse experimental models of pain, inflammation, and seizure activity.[5,34-37] Pharmacological analysis of these protective effects using ADO (P1) receptor antagonists provides mechanistic support that AK inhibition leads to increased endogenous ADO concentrations that activate different ADO receptor subtypes and is the underlying mechanism mediating the effects of AK inhibitors in vivo.[5] Importantly, systemically administered AK inhibitors were found to exert therapeutic effects (ie, anti-hyperalgesia) at 3- to 10-fold lower doses than those causing alterations in psychomotor performance (eg, exploratory motor activity or rotorod performance) and cardiovascular (eg, blood pressure and heart rate) as compared to direct-acting agonists (Table 3).

5 | SAFETY ISSUES

The preclinical profile of AK inhibitors to alleviate hyperexcitability in experimental models of seizure disorders and chronic pain without producing untoward effects on classic ADO-mediated central and peripheral endpoints provided sufficient preclinical proof of concept to advance novel AK inhibitors into early clinical development for the management of epilepsy and pain.[5] GP-3269 (Metabasis/Gensia) and ABT-702 (Abbott Laboratories) (Figure 1) are two orally bioavailable and centrally penetrant potent AK inhibitors that were considered viable clinical candidates during the late 1990s.[5] However,

TABLE 3 Potency of AK inhibitors and ADO agonists to attenuate thermal hyperalgesia and motor performance in rats

ED_{50} (μmol/kg,i.p.)					
	Thermal hyperalgesia	**Locomotor activity**	**Rotorod activity**	**Locomotor/hyperalgesia**	**Rotorod/Hyperalgesia**
ADO receptor agonist					
CPA	0.7	3	30	4.3	43
CGS 21680	1	2	>30	2	>30
NECA	0.3	0.5	7	1.7	23
AK inhibitor					
5′d-5IT	0.2	0.7	15	3.5	75
A-134974	1.0	16	>30	16	>30
ABT-702	0.7	7	>100	10	>100
A-286501	2	20	70	10	35

Note: Data from.[35] Locomotor activity, exploratory motor activity 0-30 min; Rotorod, 60 min pretreatment; Thermal Hyperalgesia, carrageenan-induced hyperalgesia.

advancement of both compounds into clinical studies was stopped at an early stage due to the discovery of compound- and mechanism-based toxicological signals.[33,39] Some of these findings for a nucleoside-based AK inhibitor were presented during an oral presentation at an international purine meeting[33] and subsequently referenced by multiple investigators[17,40-42] but elaboration of these findings and their implications for further clinical development of AK inhibitors has not been discussed previously. It is also noteworthy that the two drug discovery programs that generated these novel AK inhibitors were independently disbanded shortly after the discovery of these initial toxicology findings. Over the next decade and a half, further research on the development of AK inhibitors was largely absent until recently when new pharmacological[17,43-45] and medicinal chemistry studies of AK inhibitors have been reported.[41,46]

6 | TOXICOLOGY SUMMARY

Early toxicological studies revealed that the non-nucleoside AK inhibitor, ABT-702, possessed clastogenic activity that was idiosyncratic to this molecule but not to other members of this class of pyridopyrimidine AK inhibitors.[39,47] While not clastogenic, the clinical development of nucleoside-based AK inhibitors including clinical candidates structurally related to GP-3269 was also stopped due to toxicological signals discovered in subchronic dosing studies.[33] Histopathological analysis of tissues from 1-month toxicological studies of GP-3269 indicated the presence of brain microhemorrhage foci in rats and dogs. These effects were evidenced from both multiple-dose studies as well as after the administration of a single high dose of GP-3269 (≥100 mg/kg, p.o.). Similar toxicological endpoints were observed following dosing (1000 mg/kg, p.o.) of a structurally distinct pyridopyrimidine-derived AK inhibitor (personal communication). Importantly, these preliminary toxicology data were shared between both drug discovery groups due to the obvious safety concerns and additional studies were undertaken by each group to follow-up and confirm these data. While ABT-702 was not found to produce brain microhemorrhage foci in several single and multiple-dose toxicology studies, other structurally similar pyridopyrimidine AK inhibitors produced results similar to those observed for GP-3269. Separate experimental data indicated that both furanose and carbocyclic containing nucleoside AK inhibitors[5] produced neurovascular toxic effects similar to GP-3269, and an inactive enantiomer of one ribose-containing nucleoside AK inhibitor did not produce these signals. Additionally, in at least one experiment, systemic pre- and post-treatment of the nonselective ADO receptor antagonist, theophylline, prevented the formation of AK inhibitor-induced brain microfoci (personal communication).

These toxicological findings, while preliminary in nature, indicate that systemic administration of chemically diverse classes of potent AK inhibitors produce neurovascular toxic effects that can be evident after a single administration of a supra-therapeutic high dose. These effects were demonstrated in both rats and dogs. These effects also appear to be mechanistically related to AK inhibition since an inactive enantiomer failed to produce similar effects. However, the observed neurovascular toxic effects of AK inhibition do not seem to be attributable to the intrinsic inhibition of AK activity per se since AK inhibitor brain microhemorrhage could be blocked by administration of a nonselective ADO receptor antagonist. Collectively, these findings suggest that systemic administration of AK inhibitors increases brain ADO concentrations leading to an, as yet undefined, ADO receptor-mediated toxicity.

7 | IMPLICATIONS AND LIMITATIONS

Data are unavailable to address many of the mechanistic details (eg, relative brain penetration, pharmacokinetic parameters or kinetics of AK inhibition, and/or changes in extracellular ADO concentrations in vivo) associated with the observed toxicity of these potent and selective AK inhibitors. It also remains unknown why similar neurotoxic effects were not observed for ABT-702, yet the toxic effects of AK inhibitors appear to be a class effect since multiple different chemotypes produced similar effects. The presence of apparent vascular lesions in peripheral organs was detected in toxicology studies of ABT-702 but these effects were only present at high therapeutic multiples of plasma concentrations required for antinociception (unpublished observations). It should also be noted that genetic deletion of the AK gene in mice resulted in postnatal death of the offspring which was attributed to deleterious effects on thermoregulation, respiration, and liver toxicology.[17] Additionally, some ribose-containing AK inhibitors have also been reported to be lethal following chronic administration of antinociceptive doses in rats.[48]

The findings described above are based on the author's notes, personal communications, and the limited available literature and thus should be considered anecdotal. That said, these toxicological findings were observed by two independent groups. Both the severity of the potential harms and lack of reliable clinical biomarkers or predictability in humans led both drug discovery groups to independently discontinue further research efforts to develop AK inhibitors as therapeutic agents. Taken together, the available information regarding the toxicology readouts produced by potent and selective pharmacological blockade of AK indicates that continued optimization of these or similar molecules is unlikely to yield therapeutically useful interventions. Several groups have renewed research into the structure activity relationships of structurally novel AK inhibitors.[41,43,46] However, these new AK inhibitor chemotypes do not appear to either pharmacologically or enzymatically differentiate from the previously described highly potent AK inhibitors.[5] Furthermore, all known small molecule AK inhibitors do not differentiate between short and long forms of AK and further research is needed to determine if selective modulation of cytoplasmic or nuclear AK is biochemically feasible or would lead to physiologically important effects unrelated to downstream activation of ADO receptors.[17] One potential experimental approach to address such questions might be the further interrogation of

the recently reported quad-knockout mouse which lacks all four ADO receptors.[49] Notably, ADO and direct (subtype selective agonists) or indirect (AK inhibitor) ADO activation can lead to hypothermia.[49] This well-characterized physiologic response of ADO is abolished in the ADO receptor quad-knockout mouse.[49]

As shown in Figure 1, extracellular ADO formation can also occur via sequential nucleotide hydrolysis by CD39 and CD73. Together with the modulatory actions of AK and ADA, extracellular concentrations of ADO increase to as high as 30 μM following tissue trauma or cellular necrosis.[27] However, the diversity of purine metabolic pathways can also lead to context-specific protective or deleterious effects depending on the degree and variety of changes to individual intracellular and extracellular metabolic enzymes.[27] The function and biochemistry of the many ectonucleotidase isoforms have been expertly reviewed.[50]

Therapeutic clinical validation also known as interventional proof of concept is dependent on both confidence in the biological target coupled with confidence in the interventional strategy.[51] Drug discovery research over the last several decades has led to the generation of many potent and selective ligands for exploration of ADO-mediated cellular signaling.[6,30] However, efficient leveraging of these advances in ADO pharmacology for clinical benefit has repeatedly been hampered by the ubiquitous and fundamental involvement of ADO in virtually all physiological systems.[1,6,10,27,49,52] The findings reviewed here indicate that AK-mediated increases in extracellular ADO leading to a downstream ADO receptor activation as an interventional strategy does not appear to be therapeutically feasible. It remains unknown if selective AK modulation of intracellular biochemical mechanisms that is independent of extracellular ADO receptor activation[17] would lead to new therapeutic strategies. The findings reviewed here highlight the importance of communicating important safety issues that led to the termination of a promising drug discovery approach so that other investigators can be appropriately guided as alternative therapeutic interventions are explored.

ACKNOWLEDGMENT

The author is indebted to Dr Ken Jacobson for his valuable comments on an earlier version of this manuscript.

CONFLICT OF INTEREST

MFJ is an employee of AbbVie, Inc and holds AbbVie and Abbott stock.

ORCID

Michael F. Jarvis https://orcid.org/0000-0001-9558-8203

REFERENCES

1. Burnstock G. Purinergic nerves. *Pharmacol Rev*. 1972;24:509-581.
2. Williams M, Jarvis MF. Purinergic and pyrimidinergic receptors as potential drug targets. *Biochem Pharmacol*. 2000;59:1173-1185.
3. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci*. 2001;24:31-55.
4. Sawynok J, Liu XJ. Adenosine in the spinal cord and periphery: release and regulation of pain. *Prog Neurogibol*. 2003;69:313-340.
5. McGaraughty S, Cowart M, Jarvis MF, et al. Anticonvulsant and antinociceptive actions of novel adenosine kinase inhibitors. *Curr Top Med Chem* 2005;5:43-58.
6. Jacobson KA, Gao Z-G. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov*. 2006;5:247-263.
7. Newby AC. Adenosine and the concept of "retaliatory metabolites". *Trends Biochem Sci*. 1984;2:42-44.
8. Burnstock G. Purinergic signalling: therapeutic developments. *Front Pharmacol*. 2017;8:A-161.
9. Burnstock G. The therapeutic potential of purinergic signaling. *Biochem Pharmacol*. 2018;151:157-165.
10. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998;50:413-492.
11. Arch J, Newsholme EA. Activities and some properties of 5′-nucleotidase, adenosine kinase and adenosine deaminase in tissues from vertebrates and invertebrates in relation to the control of the concentration and the physiological role of adenosine. *Biochem J*. 1978;174:965-977.
12. Moser GH, Schrader J, Duessen A. Turnover of adenosine in plasma of human and dog blood. *Am J Physiol*. 1989;25:C799-C806.
13. Arch J, Newsholme EA. The control of the metabolism and the hormonal role of adenosine. *Essays Biochem*. 1978;14:82-123.
14. McNally T, Helfrich RJ, Cowart M, et al. Cloning and expression of the adenosine kinase gene from rat and human tissues. *Biochem Biophys Res Comm*. 1997;231:645-650.
15. Spychala J, Datta NS, Takabayashi K, et al. Cloning of human adenosine kinase cDNA: Sequence similarity to microbial ribokinases and fructokinases. *Proc Natl Acad Sci USA* 1996;93:1232–1237.
16. Matthews IL, Erion MD, Ealick SE. Structure of human adenosine kinase at 1.5a resolution. *Biochem*. 1998;10:15607-15620.
17. Boison D. Adenosine kinase: exploitation for therapeutic gains. *Pharmacol Rev*. 2013;65:906-943.
18. Hawkins CF. Bagnara AS Adenosine kinase from human erthrocytes: kinetic studies and characterization of adenosine binding sites. *Biochem*. 1987;26:1982-1987.
19. Lin BB, Hurley MC, Fox IH. Regulation of adenosine kinase by adenosine analogs. *Mol Pharmacol*. 1988;34:501-505.
20. Davies LP, Jamieson DD, Baird-Lambert JA, et al. Halogenated pyrrolopyrimidine analogs of ADO from marine organisms: pharmacological activities and potent inhibition of ADO kinase. *Biochem Pharm*. 1984;33:347-355.
21. Newby AC, Holmquist AC, Illingworth J, et al. The control of adenosine concentration in polymorphonuclear leukocytes, cultured cells and isolated perfused heart from the rat. *Biochem J*. 1983;214:317-323.
22. Engler RL. Consequences of activation and ADO mediated inhibition of granulocytes during myocardial ischemia. *Fed Proc*. 1987;46:2407-2412.
23. Britton DR, Mikusa J, Lee C-H, Jarvis MF, Williams M, Kowaluk EA. Site and event specific increase of striatal adenosine release by adenosine kinase inhibition in rats. *Neurosci Lett*. 1999;266:93-96.
24. Pak MA, Haas HL, Decking U, Schrader J. Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacol*. 1994;33:1049-1053.
25. Golembiowska K, White TD, Sawynok J. Adenosine kinase inhibitors augment release of adenosine from spinal cord slices. *Eur J Pharmacol*. 1996;307:157-162.
26. Liu XJ, White TD, Sawynok J. Potentiation of formalin-evoked adenosine release by an adenosine kinase inhibitor and an adenosine

deaminase inhibitor in the rat hind paw: a microdialysis study. *Eur J Pharmacol*. 2000;408:143-152.
27. Chen J-F, Eltzchig HK, Fredholm BB. Adenosine receptors as drug targets – what are the challenges? *Nat Rev Drug Discov*. 2013;12:265-285.
28. Mullane K, Young M. Acadesine: Prototype adenosine regulating agent for treating myocardial ischemia-reperfusion injury. *Drug Dev Res*. 1993;23:336-343.
29. Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. *Expert Opin Ther Drugs*. 2000;9:1-13.
30. Jacobson KA, Müller CE. Medicinal chemistry of adenosine, P2Y and P2X receptors. *Neuropharmacol*. 2016;104:31-49.
31. Lee C-H, Jiang M, Cowart M, et al. Discovery of 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-d]pyrimidine, an orally active, non-nucleoside adenosine kinase inhibitor. *J Med Chem*. 2001;44:2133-2138.
32. Erion MD, Ugarkar BG, DaRe J, et al. Design, synthesis and anticonvulsant activity of the potent adenosine kinase inhibitor GP-3269. *Nucleosides Nucleotides*. 1997;16:1013-1021.
33. Erion MD, Wiesner JB, Rosengren S, et al. A therapeutic potential of adenosine kinase inhibitors as analgesic agents. *Drug Dev Res*. 2000;50:S14-S16.
34. Jarvis MF, Yu H, Kohlhaas K, et al. ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties: I. In vitro characterization and acute antinociceptive effects in the mouse. *J Pharmacol Exp Ther* 2000;295:1156-1164.
35. Jarvis MF, Mikusa J, Chu KL, et al. Comparison of the ability of adenosine kinase inhibitors and adenosine receptor agonists to attenuate thermal hyperalgesia and reduce motor performance in rats. *Pharm Biochem Behav*. 2002;73:573-581.
36. Muchmore SW, Smith RA, Stewart AO, et al. Crystal structures of human adenosine kinase inhibitor complexes reveal two distinct binding sites. *J Med Chem*. 2006;49:6726-6731.
37. Kowaluk EA, Mikusa J, Wismer CT, et al. ABT-702, a novel orally effective adenosine kinase (AK) inhibitor analgesic with anti-inflammatory properties. II. antinociceptive and anti-Inflammatory effects in rat models of persistent, inflammatory, and neuropathic pain. *J Pharmacol Exp Ther*. 2000;295:1165-1174.
38. Wiesner JB, Ugarkar BG, Castellino AJ, et al. Adenosine kinase inhibitors as a novel approach to anti-convulsant therapy. *J Pharmacol Exp Ther*. 1999;289:1669-1677.
39. Matulenko MA, Lee C-H, Jiang M. 5-(3-Bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido[2,3-d]pyrimidin-4-ylamine: Structure-activity relationships of 7-substituted heteroaryl analogs as non-nucleoside, orally active adenosine kinase inhibitors. *Bioorg Med Chem*. 2005;13:3705-3720.
40. McGaraughty S, Cowart M, Jarvis MF. Recent developments in the discovery of novel adenosine kinase inhibitors: mechanism of action and therapeutic potential. *CNS Drug Rev*. 2001;7:415-432.
41. Toti KS, Osborne D, Ciancetta A, et al. South (S) and north (N)-methanocarba-7-deazaadenodine analogues as inhibitors of human adenosine kinase. *J Med Chem*. 2016;59:6860-6877.
42. Sawynok J. Adenosine receptors for pain. *Neurosci*. 2016;338:1-18.
43. Kohler D, Streibenberger A, Morote-Garcia JC, et al. Inhibition of adenosine kinase attenuates acute lung injury. *Crit. Care Med*. 2016;44:181-189.
44. Wahlman C, Doyle TM, Little JW, et al. Chemotherapy-induced pain is promoted by enhanced spinal adenosine kinase levels through astrocyte-dependent mechanisms. *Pain*. 2018;159:1025-1033.
45. Zhu CZ, Gopalakrishnan S, Doyle K, et al. A-306989, an inhibitor of adenosine kinase is renoprotective in rodent models of podocyte, basement membrane and obstructive injury. *Eur J Pharmacol*. 2016;788:1-11.
46. Kose M, Schiedel AC, Bauer AA, et al. Focused screening to identify new adenosine kinase inhibitors. *Bioorg. Med. Chem*. 2016;24:5127-5133.
47. Matulenko M, Paight E, Frey R. 4-Amino-5-aryl-6-arylethynylpyrimidines: structure-activity relationships on non-nucleoside adenosine kinase inhibitors. *Bioorg Med Chem Lett*. 2007;15:1586-1605.
48. Bookser BC, Ugarkar BG, Matelich MC. Adenosine kinase inhibitors. 6. synthesis, water solubility, and antinociceptive activity of 5-phenyl-7-(5-deoxy-β-D-ribofuranosyl) pyrrolo[2,3-d]pyrimidines substituted at C4 with glycinamides and related compounds. *J Med Chem*. 2005;48:7808-7820.
49. Xiao C, Liu N, Jacobson KA, et al. Physiology and effect of nucleosides in mice lacking all four adenosine receptors. *PLOS Bio*. 2019;17:e3000161.
50. Zimmermann H, Zebisch M, Strater N. Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Sig*. 2012;8:437-502.
51. Vicini P, van der Graaf PH. Systems pharmacology for drug discovery and development: Paradigm shift or flash in the pan? *Clin Pharm Ther*. 2013;93:379-381.
52. Cunha RA. Signaling by adenosine receptors – homeostatic or allostatic control? *PLOS Bio*. 2019;17:e3000213.

How to cite this article: Jarvis MF. Therapeutic potential of adenosine kinase inhibition—Revisited. *Pharmacol Res Perspect*. 2019;e00506. https://doi.org/10.1002/prp2.506