1521-0081/73/3/1150–1171$35.00
PHARMACOLOGICAL REVIEWS

https://doi.org/10.1124/pharmrev.120.000122
Pharmacol Rev 73:1150–1171, July 2021

ASSOCIATE EDITOR: MICHAEL GOTTESMAN

Aldo-Keto Reductases and Cancer Drug Resistance

Trevor M. Penning, Sravan Jonnalagadda, Paul C. Trippier, and Tea Lanišnik Rižner

Center of Excellence in Environmental Toxicology, Department of Systems Pharmacology & Translational Therapeutics, Philadelphia, Pennsylvania (T.M.P.); Department of Pharmaceutical Science (S.J., P.C.T.) and Fred and Pamela Buffett Cancer Center (P.C.T.), University of Nebraska Medical Center and UNMC Center for Drug Discovery, Omaha, Nebraska; and Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia (T.L.R.)

***Abstract*——Human aldo-keto reductases (AKRs) catalyze the NADPH-dependent reduction of carbonyl groups to alcohols for conjugation reactions to proceed. They are implicated in resistance to cancer chemotherapeutic agents either because they are directly involved in their metabolism or help eradicate**

Address correspondence to: Dr. Trevor M. Penning, Department of Systems Pharmacology & Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, 1315 BRBII/IIII, 421 Curie Blvd. Philadelphia, PA 19104. E-mail: penning@upenn.edu

These studies were supported in part by National Institutes of Health [Grants P30-ES013508 and R01-ES029294] (to T.M.P.) and [Grant R01-CA226436] (to P.C.T.) and by the Slovenian Research Agency [Grants J3-8212 and J3-2535] (to T.L.R.).
https://doi.org/10.1124/pharmrev.120.000122

the cellular stress created by these agents (e.g., reactive oxygen species and lipid peroxides). Furthermore, this cellular stress activates the Nuclear factor-erythroid 2 p45-related factor 2 (NRF2)-Kelch-like ECH-associated protein 1 pathway. As many human *AKR* genes are upregulated by the NRF2 transcription factor, this leads to a feed-forward mechanism to enhance drug resistance. Resistance to major classes of chemotherapeutic agents (anthracyclines, mitomycin, *cis*-platin, antitubulin agents, vinca alkaloids, and cyclophosphamide) occurs by this mechanism. Human AKRs also catalyze the synthesis of androgens and estrogens and the elimination of progestogens and are involved in hormonal-dependent malignancies. They are upregulated by antihormonal therapy providing a second mechanism for cancer drug resistance. Inhibitors of the NRF2 system or pan-AKR1C inhibitors offer promise to surmount cancer drug resistance and/or synergize the effects of existing drugs.

***Significance Statement*——Aldo-keto reductases (AKRs) are overexpressed in a large number of human tumors and mediate resistance to cancer chemotherapeutics and antihormonal therapies. Existing drugs and new agents in development may surmount this resistance by acting as specific AKR isoforms or AKR pan-inhibitors to improve clinical outcome.**

I. Introduction to Aldo-Keto Reductases

Aldo-keto reductases (AKRs) are a superfamily of NAD(P)(H)-dependent oxidoreductases, which are predominately cytosolic and most often exist as monomers of 37 kDa in molecular weight. In most instances, they catalyze the reduction of carbonyl groups to alcohols. Aldehydes are converted to primary alcohols and ketones are converted to secondary alcohols on a variety of substrates (Jez and Penning, 2001; Mindnich and Penning, 2009). This functionalization would categorize these enzymes as phase-1 enzymes. Upon functionalization, the alcohols can be conjugated as glucuronides or as sulfates for elimination.

There are 15 human AKRs, and while their carbonyl substrates may be diverse, there are some common features in these proteins (Penning and Drury, 2007) (Table 1). They share a common $(\alpha/\beta)_8$ barrel in which there is an alternating pattern of α-helices and β-strands. The β-strands make up the staves of a barrel, and at the base of the barrel a catalytic tetrad exists of Y55, K84, D40, and H117, the exception being AKR1D1, in which a H120E substitution exists to replace the catalytic histidine. At the back of the barrel, three large loops exist which help define substrate specificity, where different amino acids are recruited to the binding pocket based on substrate (Fig. 1).

AKRs are overexpressed in a broad range of human cancers, e.g., prostate (Stanbrough et al., 2006), breast (Ma et al., 2012; McNamara and Sasano, 2019), glioma (Park et al., 2010), neuroblastoma (Lyon et al., 2013), nonsmall lung cancer and small cell lung cancer (Hsu et al., 2001; Tian et al., 2016), colon cancer (Kropotova et al., 2014; Zu et al., 2017), cancer of the small intestine (Wang et al., 2009), and acute myelocytic leukemia (AML) (Birtwistle et al., 2009). Tumor-specific expression of the AKR isoforms has been observed, e.g., nonsmall cell lung cancer (*AKR1C1, AKR1C2, AKR1C3* and *AKR1B10*) (Hsu et al., 2001; Cubillos-Tian et al., 2016; Angulo et al., 2020), colon cancer (*AKR1B10*) (Kropotova et al., 2014), breast and prostate cancer (*AKR1C3*) (Stanbrough et al., 2006; McNamara and Sasano, 2019), and AML (*AKR1C3*) (Birtwistle et al., 2009). The abundance of *AKR* overexpression has led to the proposition that they can be used as tumor biomarkers (Hamid et al., 2013; Tian et al., 2014; Tian et al., 2016; Cubillos-Angulo et al., 2020).

The AKRs are also stress response genes and respond to electrophilic and oxidative stress, osmotic stress, and steroid hormones (Penning and Drury 2007). They are induced by the Nuclear factor-erythroid 2 p45-related factor 2 (NRF2) (*NFE2L2*)-Kelch-like ECH-associated protein 1 (KEAP1) (*KEAP1*) pathway (Tebay et al., 2015) by AP-1 transcription factors, a tonictiy responsive enhancer (Ko et al., 1997), and are regulated by steroid hormone receptors via steroid response elements (Penning and Drury 2007). Since they respond to electrophilic and oxidative stress stimuli, which are generated by chemotherapeutic agents, they are upregulated by these agents, and this contributes to drug resistance. Because they also play a role in steroid hormone transformation, they are involved in chemoresistance to hormone ablative therapies.

II. Stress Response and Nuclear Factor-Erythroid 2 p45-Related Factor 2 Induction of Aldo-Keto Reductases

NRF2-KEAP1 is a master regulator of stress response genes (Tebay et al., 2015). Under basal conditions, NRF2 is tethered to KEAP1, which is an adaptor protein for the cullin-3. RealIy Interesting New Gene-box protein 1 ubiquitin ligase, which targets NRF2 for ubiquitination and proteasomal degradation (Dinkova-Kostova et al., 2005). Electrophiles and reactive oxygen

ABBREVIATIONS: ABC, ATP-binding cassette; AKR, aldo-keto reductase; AKT, protein kinase B; ALDH, aldehyde dehydrogenase; AML, acute myelocytic leukemia; AR, androgen receptor; ARSI, androgen receptor signaling inhibitor; araC, cytarabine; ARE, antioxidant response element; ARN, apalutamide; ARSI, androgen receptor signaling inhibitor; COX, cyclooxygenase; DGR, double glycine repeat; Dox, doxorubicin; GSH, glutahione; HaCaT, immortal human keratinocyte cell line; 4-HNE, 4-hydroxy-2-nonenal; INDO, indomethacin; IVR, intervening region; KEAP1, Kelch-like ECH-associated protein 1; NADPH, nicotinamide adenine dinucleotide phosphate; NQO1, NADPH: quinone oxidoreductase; NRF2/NFE2L2, nuclear factor-erythroid 2 p45-related factor 2; NSAID, nonsteroidal anti-inflammatory drug; ODM, darolutamide; PPARγ, peroxisome proliferator activated receptor γ; PI3K, phosphoinositide 3–kinase; P450, cytochrome P450; ROS, reactive oxygen species; si-RNA, short interfering RNA; SLC, solute carrier; T-ALL, T-cell acute lymphoblastic leukemia; TMPRSS2-ERG, transmembrane proetase serine 2 and eryrthroblast transformation specific related gene/protein.

TABLE 1
Human AKRs and involvement in drug resistance

Gene	Protein Names (Aliases)	Chromosomal Localization	Substrate Specificity
AKR1A1	Aldehyde reductase; dihydrodiol dehydrogenase 1	1p33-p32	*DL*-glyceraldehyde Melvadate
AKR1B1	Aldose reductase	7q35	Glucose, advanced end glycation products, 4-HNE, reactive carbonyls
AKR1B10	Small intestine like aldose reductase; 9-cis-retinal reductase	7q33	Retinal; reactive carbonyls
AKR1B15	3-Keto- acyl CoA reductase	7q33	3-Keto-acyl-coA; 9-*cis*-retinaldehyde
AKR1C1	3α(20α)-Hydroxysteroid dehydrogenase; dihydrodiol dehydrogenase 1;	10p15-10p14	Reduction of 20-ketosteroids e.g., progesterone
AKR1C2	3α-hydroxysteroid dehydrogenase type 3; dihydrodiol dehydrogenase 2; bile-acid binding protein	10p15-10p14	Reduction of 3-ketosteroids, dihydroprogesterone, dihydrotestosterone
AKR1C3	3α-hydroxysteroid dehydrogenase type 2; 17β-hydroxysteroid dehydrogenase type 5; prostaglandin F synthase; dihydrodiol dehydrogenase X	10p15-10p14	Reduction of 17-ketosteroids; 4-androstene-3,17-dione; estrone; prostaglandin H_2; prostaglandin D_2
AKR1C4	3α−hydroxysteroid dehydrogenase type 1; dihydrodiol dehydrogenase 4; chlordecone reductase	10p15-10p14	3-Ketosteroids; bile acid precursors; chlordecone
AKR1D1	Steroid 5β-reductase	7q32-7q33	Delta^4-3-Ketosteroids
AKR1E2	1,5-Andyhro-D-frucose reductase	10p15	1,5-anhydro-D-fructose
AKR6A3	Potassium voltage gated channel β-subunit-1	3q26.1	
AKR6A5	Potassium voltage gated channel β-subunit-2	1p36.3	
AKR6A9	Potassium voltage gated channel β-subunit-3	17p13.1	
AKR7A2	Aflatoxin aldehyde reductase	1p35.1-p36.23	Reduction of succinic semialdehyde; acrolein 2-carboxybenzlaldehyde; aflatoxin dialdehyde
AKR7A3	Aflatoxin aldehyde reductase	1p35.1-p36.23	Aflatoxin dialdehyde

species (ROS) modify key cysteine residues in KEAP1, which prevents ubiquitination of NRF2 (Wakabayashi et al., 2004; Dinkova-Kostova et al., 2017). Newly synthesized NRF2 can thus evade ubiquitination, translocate to the nucleus, and bind to its heterodimeric partner small-maf on the antioxidant response element (ARE) (5′-A/G-TGA-C/G-NNN-GC-A/G-3′) of stress response genes to increase gene transcription (Fig. 2). *AKR1C1-AKR1C3* genes contain multiple AREs (6–16 based on a consensus sequence search and a positional matrix search) in their promoters (Tebay et al., 2015) (see Table 2).

Prototypic NRF2 activators such as *R*-sulforaphane (SFN) and the triterpenoid 1-(2-Cyano-3,12,28-trioxooleana-1,9(11)-dien-28-yl)-1*H*-imidazole (CDDO) have been used as cancer chemo-prevention agents in that they could lead to the inactivation of electrophiles that would damage DNA and initiate cancer (Yates et al., 2006; Kensler et al., 2013). The same activators could also lead to the elimination of cytotoxic species required for the efficacy of the cancer chemotherapeutic response. Thus, the activation of NRF2 leading to chemoresistance is considered the "dark side" of NRF2 activation.

Studies by Ciaccio and Tew originally showed that human colon cancer cells that were resistant to ethacrynic acid had highly elevated levels of dihydrodiol dehydrogenase (Ciaccio et al., 1993; Ciaccio and Tew, 1994; Ciaccio et al., 1994), which at that time was a trivial name given to AKR1C1-AKR1C4 (Burczynski et al., 1998). Further, Northern analysis and RNA protection studies showed that *AKR1C1-AKR1C3* transcripts were upregulated by electrophilic and oxidative stress in HepG2 cells (Burczynski et al., 1999) (Table 2). MacLeod et al. (2009) showed that small interfering-RNA (si-RNA) for *KEAP1* in human immortal keratinocytes (HaCaT) resulted in upregulation of *AKR1B10, AKR1C1/2,* and *AKR1C3* transcripts by reverse-transcription polymerase chain reaction and AKR1B10 and AKR1C1/2 protein. Similarly, *NFE2L2* si-RNA resulted in downregulation of the same AKRs. In another study, it was found that *KEAP1* si-RNA resulted in upregulation of *AKR1B10, AKR1C1, AKR1C2*, and *AKR1C3* in human breast cancer cells at the transcript level and at the protein level as well by stable isotope labeling by amino acids in cell culture-labeling followed by mass spectrometry. Furthermore, this transcriptomic and proteomic analysis showed that these genes were the most highly regulated by the NRF2-KEAP1 pathway (Agyeman et al., 2012). Recent CRISPR/Cas9 gene editing of *NFE2L2* in A549 lung adenocarcinoma cells showed that high constitutive expression of AKR1C1,

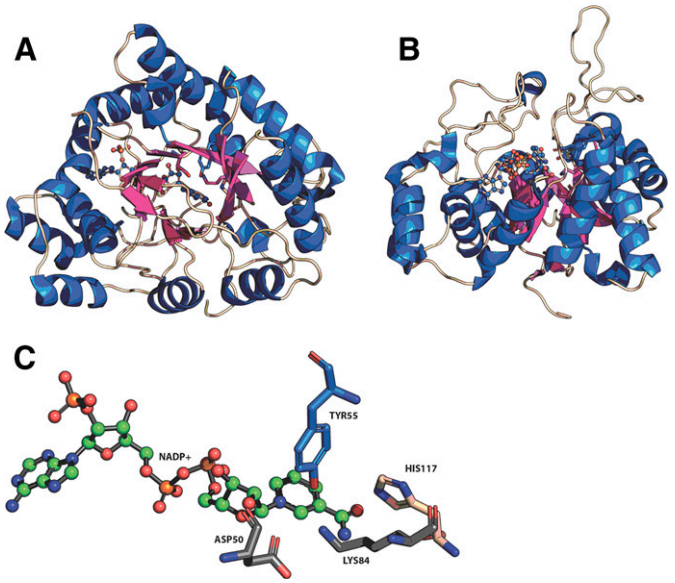

Fig. 1. Structure of human AKRs and catalytic tetrad. α/β_8-barrel protein fold of AKRs (A); loop structures at back of barrel (B); and (C) catalytic tetrad. From AKR1C2.NADP$^+$ complex (PDB 2hdj). Helices (blue); β-strands (purple); stick model shows position of NADP$^+$ and catalytic tetrad in A, and B. Figure produced in Pymol.

AKR1C2, and AKR1C3 was eliminated by NRF2 deletion (Murray et al., 2019b). In A549 cells, *KEAP1* is hypermethylated and mutated, leading to high levels of NRF2 (Wang et al., 2008; Fabrizio et al., 2019). It is now known that the majority of the human *AKR* genes are regulated by the NRF2-KEAP1 pathway and these data are summarized in Table 2. These findings suggest that NRF2 activation would exacerbate drug resistance mediated by AKRs. In addition, NRF2 activation can lead to an increase in GSH biosynthesis via induction of gamma-glutamyl cysteine synthetase light chain and increase cofactor availability for glutathione-*S*-transferases (Dinkova-Kostova et al., 2005). Increased glutathione-*S*-transferase activity can eliminate cytotoxic species generated by chemotherapeutic agents (e.g., 4-hydroxy-2-nonenal and acrolein) by glutathione conjugation. Thus, a place exists for both AKR inhibitors and inhibitors of NRF2 to surmount drug resistance. Examples of how AKRs mediate resistance to major classes of chemotherapeutic drugs and antihormonal therapies follow in sections 4 and 5.

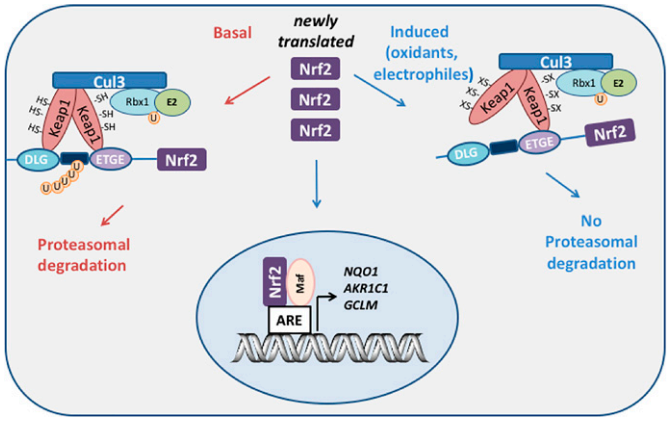

Fig. 2. NRF2-Keap1 regulation of human AKR genes. Under basal conditions, the transcription factor Nrf2 is sequestered by Keap1 which acts as an adaptor program for culin 3 (cul 3) ubiquitin ligase which leads to the ubiquitination of Nrf2 and its proteasomal degradation. Upon exposure to oxidative or electrophilic stress, cysteine residues on Keap1 become either oxidized or covalently modified which disrupts the interaction with Nrf2 and prevents its ubiquitination. Under these conditions, newly synthesized Nrf2 evades proteasomal degradation, translocates to the nucleus where it binds to its heterodimeric partner small maf; and the resulting dimer binds to ARE in the promoters of genes that detoxify the stress signal, e.g., AKR1C, NQO1, and γ-glutamylcysteine synthetase (GCLM).

III. Somatic Mutations and Epigenetic Regulation of the Nuclear Factor-Erythroid 2 p45-Related Factor 2-Kelch-Like ECH-Associated Protein 1 Pathway

Somatic mutations in *KEAP1* that suppress its activity or epigenetic regulation of the NRF2-KEAP1 pathway could lead to high constitutive expression of *AKR*s and could contribute to cancer drug resistance in multiple tumor types.

There are 174 *KEAP1* somatic mutations in human lung cancer which may affect the expression of NRF2. They reside in the intervening region (IVR) domain responsible for the ubiquitination of NRF2, and in the double glycine repeat (DGR) domain which interacts with NRF2 (Fig. 3) (Canning et al., 2015; Grossman et al., 2016). These mutations are often observed in different lung cancer subtypes: for example, the A427V mutant (located in the DGR domain of KEAP1) is seen in small cell lung carcinoma (Ohta et al., 2008), the H200P and R272C mutants (located in the IVR domain of KEAP1) are observed in adenocarcinoma (Ohta et al., 2008), and the G430C, R415G, and G364C mutants (located in the DGR domain of KEAP1) are seen in adenocarcinoma. Early detection of *KEAP1* mutations in different subtypes of lung cancer may predict early onset insensitivity to chemotherapeutic agents and could be clinically actionable. Somatic mutations of *NFE2L2* in the coding region have also been observed in human cancers (Kerins et al., 2018). NRF2 mis-sense mutations occur in amino acids in the DLG or ETGE motifs which interact with KEAP1, resulting in aberrant cellular accumulation of NRF2. Cells expressing NRF2 mutants display constitutive induction of cytoprotective enzymes and are insensitive to KEAP1-mediated regulation.

Mutations of *KEAP1* and *NFE2L2* occur in more than 30% of lung cancer cases and may contribute to drug resistance. The Cancer Genome Atlas database catalogs 226 unique *NFE2L2*-mutant tumors identified from 10,364 cancer cases (Kerins et al., 2018). *NFE2L2* mutations were found in 21 out of 33 different tumor types. A total of 11 hotspots were

TABLE 2
Evidence that human aldo-keto reductases are regulated by the NRF2-KEAP1 system

			Increased Expression with NRF2 Activator				Decreased Expression with *NFE2L2*/*KEAP1* k/d			
AKR Gene	AREs in Promotor Region	AREs Based on Positional Matrix	Inducer	Increased RNA	Increased Protein/Activity	Cell Type*	Knockdown Method	Change in RNA	Change in Protein/ Activity	Cell Type*
AKR1A1	6	5					*KEAP1* sh-RNA	yes		HK-2[f]
							KEAP1 si-RNA	no		HaCaT[g]
							NFE2L2 si-RNA	yes		HaCaT[g]
AKR1B1	1	3					*KEAP1* si-RNA	yes	yes	MCF-10A[a]
							KEAP1 sh-RNA	yes		HK-2[f]
							KEAP1 si-RNA	yes		HaCaT[g]
							NFE2L2 si-RNA	yes		HaCaT[g]
AKR1B10	4	2	SFN	yes	yes	MCF10A[a]	*KEAP1* si-RNA	yes	yes	MCF-10A[a]
				yes	yes	MCF12A[a]	*KEAP1* si-RNA	yes	yes	MCF-12A[a]
							KEAP1 sh-RNA	yes		HK-2[f]
							KEAP1 si-RNA	yes	yes	HaCaT[g]
							NFE2L2 si-RNA	yes		HaCaT[g]
AKR1B15	4	5								
AKR1C1	10	16	Ethacrynic acid,	yes	yes	HT29[b]	*KEAP1* si-RNA	yes	yes	MCF-10A[a]
			SFN, CDDO	yes	yes	HepG2[c]	*KEAP1* si-RNA	yes	yes	MCF-12A[a]
				yes	yes	MCF10A[a]	*KEAP1* sh-RNA	yes		HK-2[f]
				yes	yes	MCF12A[a]	*KEAP1* si-RNA	yes	yes	HaCaT[g]
				yes	yes	HBEC-KT[d]	*NFE2L2* si-RNA	yes	yes	HaCaT[g]
								yes	yes	HK-2[f]
							CRISPR/Cas9 *NFE2L2*			A-549[d]
AKR1C2	15	24	Ethacrynic acid,	yes	yes	HT29[b]				
			SFN, CDDO	yes	yes	HepG2[c]	*KEAP1* si-RNA	yes	yes	MCF-10A[a]
				yes	yes	MCF10A[a]	*KEAP1* si-RNA	yes	yes	MCF-12A[a]
				yes	yes	MCF12A[a]	*KEAP1* si-RNA	no	yes	HaCaT[g]
				yes	yes	HBEC-KT[d]	*KEAP1* sh-RNA	yes		HK-2[f]
							NFE2L2 si-RNA	no	no	HaCaT[g]
								yes	yes	HK-2[f]
							CRISPR/Cas9 *NFE2L2*			A-549[d]
AKR1C3	4	6	Ethacrynic acid,	yes	yes	HepG2[c]	*KEAP1* si-RNA	yes	yes	MCF-10A[a]
			SFN, CDDO	yes	yes	MCF10A[a]	*KEAP1* si-RNA	yes	yes	MCF-12A[a]
				yes	yes	MCF12A[a]	*KEAP1* sh-RNA	yes		HK-2[f]
				yes	yes	HBEC-KT[d]	*KEAP1* si-RNA	yes		HaCaT[g]
							NFE2L2 si-RNA	yes		HaCaT[g]
							CRISPR/Cas9 *NFE2L2*	yes	yes	A-549[d]
AKR1C4	2	2								
AKR1D1	11	27								
AKR1E2	6	3								
AKR7A2	4	9	Methyl gloxal	yes	yes	HepG2[e]	*NFE2L2* si-RNA	yes	yes	Hep-G2[e]
AKR7A3	1	4								

[a]Agyeman et al. (2012).
[b]Ciaccio et al. (1994).
[c]Burczynski et al. (1999).
[d]Murray et al. (2019).
[e]Li et al. (2015).
[f]Jung (2013).
[g]MacLeod (2009).
*Cell lines: HK-2 (renal carcinoma); MCF-10A, MCF-12A (breast carcinoma); A-549 (lung adenocarcinoma); Hep-G2 (hepatoma cells); HaCaT (human keratinocytes).

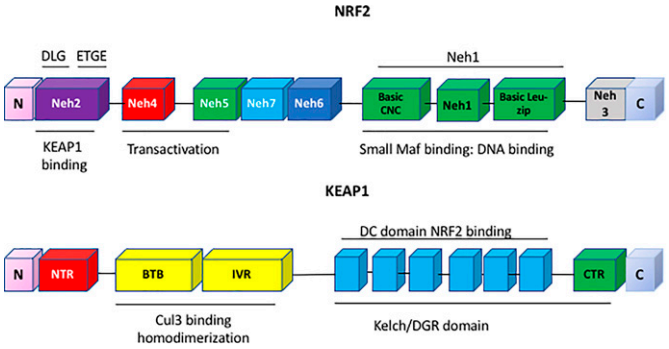

Fig. 3. Domain structures of KEAP1 and NRF2. KEAP1 has four domains, the N-terminal Broad complex, Tramtrack and Bric-à-Brac (BTB) domain which binds cullin 3 ubiquitin ligase, an IVR, and the Kelch domain which binds NRF2. NRF2 contains seven functional domains, Neh1–7. Neh1 contains the DNA binding and heterodimerization domain for small maf, while Neh2 is the regulatory domain which interacts with Keap1 via its DLG and ETGE motifs.

identified. Of these, amino acids in the DLG motif R25 to R34 were among the common hotspots observed.

The NRF2-KEAP1 pathway is also epigenetically regulated by DNA methylation, histone modification, and long non-coding-RNA (Guo et al., 2015). The methylation pattern of CpG islands in the *KEAP1* promoter differ between normal lung and lung cancer and between normal human bronchial epithelial cells and human lung carcinoma cells (Fabrizio et al., 2019), and similar differences were noted in colorectal cancer cells and tissues (Hanada et al., 2012). Treatment of lung cancer cells with the methylation inhibitor 5′-aza-2′-deoxycytidine restored *KEAP1* mRNA levels, suggesting an epigenetic mechanism of *KEAP1* regulation. Thus, hypermethylation of *KEAP1* leads to its suppression. It is unknown if the methylation status of *KEAP1*, and hence its regulation, is affected by chronic chemotherapeutic drug exposure to impose epigenetic memory.

IV. Resistance to Major Cancer Chemotherapeutics Mediated by Aldo-Keto Reductases

Chemotherapeutic drug resistance is a major mechanism by which tumors can evade drug treatment. Resistance is multifactorial and can include increased drug metabolism, e.g., induction of *CYP3A4* (Yao et al., 2000), changes in expression of solute uptake transporters [solute carriers (SLC)] and drug efflux pumps, e.g., ABC transporters (P-glycoprotein and multiple drug resistance proteins) (Gupta et al., 2020), and increased GSH biosynthesis to increase conjugation capacity by glutathione-*S*-transferases and counter oxidative stress. SLC are less studied as compared with ABC transporters, although about 70% of cytotoxic compounds depend on SLC transporters (Lanthaler et al., 2011). As uptake transporters, they regulate influx of drugs (e.g., SLC29A1 mediates the influx of fluorouracil, SLC35A2 and SLC38A5 mediate the influx of *cis*-platin, and more than 20 SLC mediate the uptake of methotrexate) and affect their intracellular concentrations. The role of these transporters was identified by genetic screens against 60 different cytotoxic drugs (Girardi et al., 2020). Downregulation of *SLC* genes may prevent drug uptake, and upregulation may support higher energy and nutrition requirement of cancer cells. Alterations in expression of a number of *SLC* genes has been observed in ovarian cancer cell lines W1 and A2780 resistant to *cis*-platin, doxorubicin, topotecan, and paclitaxel (Januchowski et al., 2013, 2014). Since multiple mechanisms of drug resistance can exist, treatment of resistance by targeting a single mechanism is likely to fail since, under the selection pressure of drug treatment, the tumor can adapt and substitute other pathways of resistance.

Numerous studies implicate human AKRs in drug resistance to the major classes of cancer chemotherapeutic agents: the anthracyclines (daunorubicin and doxorubicin); mitomycin; *cis*-platin, methotrexate, oracin, vinca alkaloids, and cyclophosphamide. Three mechanisms may account for the drug resistance observed: 1) involvement of AKRs in drug inactivation by catalyzing carbonyl reduction to alcohols; 2) involvement of AKRs in the reduction of quinones to hydroquinones (anthracyclines and mitomycin) with the concomitant production of ROS to increase NRF2 activation and elevate AKR expression by a feed-forward mechanism; and 3) upregulation due to the cellular stress created by drug exposure and elimination of that stress, e.g., inactivation of 4-hydroxy-2-nonenal (4-HNE) produced as a byproduct of lipid peroxidation (Fig. 4). When five human recombinant AKRs were compared for the conversion of 4-HNE to 1,4-dihydroxy-2-nonene, the enzyme with the highest catalytic efficiency for this reaction was found to be AKR1C1 (Burczynski et al., 2001). Direct evidence that these AKRs are involved in drug resistance comes from genetic approaches, e.g., RNA interference, transfection studies, and pharmacological inhibition of AKRs. While overexpression of the AKRs is commonly mediated by the NRF2-KEAP1 pathway, they can also be overexpressed as part of disease pathogenesis. In the subsections that follow, we describe the mode-of-action of each therapeutic agent, evidence that AKRs are involved in resistance to that drug based on enzyme activity, transfection studies, forced overexpression, RNA interference, and association studies with overexpression in cancer cell lines. Where possible, we address the relative contribution of AKRs to the drug resistance phenotype. The ability of AKR isoform inhibitors to reverse drug resistance and

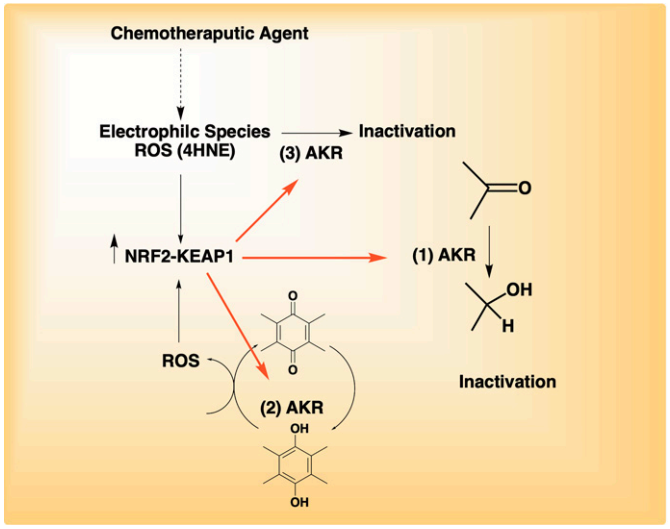

Fig. 4. Drug resistance mechanisms mediated by AKRs. In mechanism 1, AKRs inactivate carbonyls on chemotherapeutic agents adding in their elimination; in mechanism 2, AKRs redox cycle *p*-quinones and produce ROS to activate NRF2 to increase AKR gene expression; in mechanism 3, AKRs inactivate breakdown cytotoxic products of lipid peroxides such as 4-HNE.

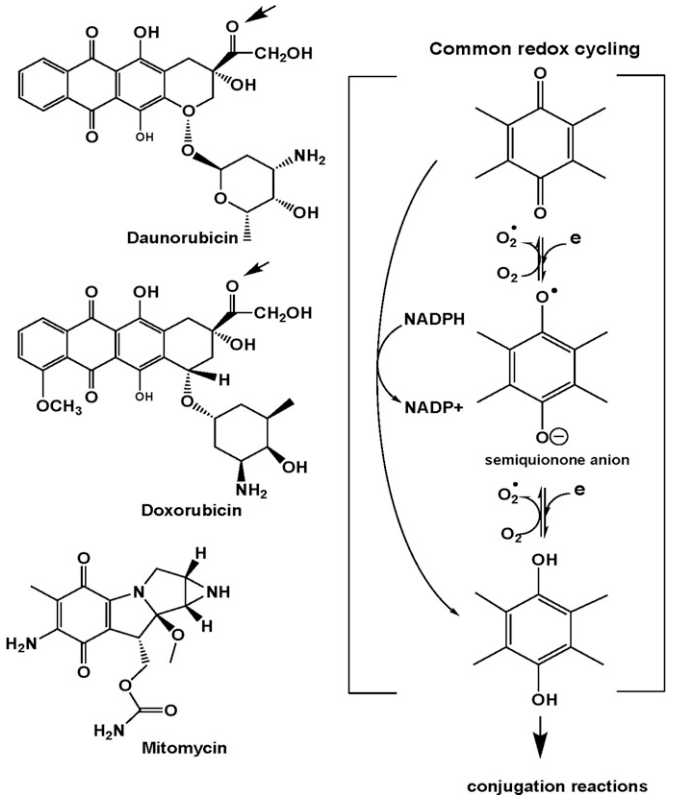

Fig. 5. Structures of daunorubicin and doxorubicin (representative anthracyclines) and mitomycin inactivated by AKRs. Arrow on structures on left show the carbonyl group reduced and the common *p*-quinone structure. Redox-cycling (right) shows the formation of ROS that could result in the formation of cytotoxic 4-HNE that would be inactivated by AKRs, and the formation of the hydroquinone which can be eliminated as a conjugate.

synergize the effects of chemotherapeutic drugs are described in sections 7 and 8, respectively.

A. Anthracyclines (Daunorubicin, Doxorubicin, Idarubicin, and Epirubicin)

The anthracycline cancer chemotherapeutic agents intercalate double-stranded DNA and inhibit transcription and replication. They form a heterotrimeric complex with topoisomerase II and DNA. Topoisomerase catalyzes double-strand DNA breaks when uncoiling supercoiled DNA, and the ternary drug complex prevents religation of the broken strands essential for DNA replication (Gewirtz, 1999). The AKRs can eliminate the anthracyclines via carbonyl reduction. The anthracyclines also possess a *p*-quinone in their structure which can undergo one electron and two electron redox cycling leading to the production of ROS and oxidative stress (Fig. 5). The production of ROS leads to induction of the *AKR* genes which can eliminate both the anthracycline and the 4-HNE produced as a result of ROS.

Aldehyde reductase (AKR1A1) was first identified as "daunorubicin reductase" by Felsted et al. (1974). This activity was important since it eliminated the active drug by forming daunorubicinol, but in doing so formed this cardiotoxic metabolite. Thus, AKR1A1 inhibitors would be chemoprotective and promote chemosensitization (Fig. 5). The involvement of carbonyl reduction in anthracycline drug resistance was supported by transfection studies from the Maser group, which showed that overexpression of carbonyl reductase, *AKR1A1*, and *AKR1B1* were involved in drug resistance in human pancreatic cancer cells (Plebuch et al., 2007). AKRs have since been implicated in resistance to a wide range of anthracyclines including daunorubicin, doxorubicin, idarubicin, and epirubicin.

Daunorubicin now appears to be inactivated by AKR1A1, AKR1C2, and AKR1B1 (Ohara et al., 1995; Plebuch et al., 2007a) and AKR1C3 (Hofman et al., 2014). Examination of catalytic efficiencies showed that AKR1C3 had the highest catalytic efficiency for the reduction of daunorubicin, while reduction of doxorubicin and idarubicin occurred with 2- to 5-fold lower catalytic efficiencies, respectively (Hofman et al., 2014). A dose-dependent relationship existed between the level of AKR1C3 expressed and the ensuing drug resistance observed. AKR1B10 also reduces daunorubicin and idar ubicin but is less active toward doxorubicin and epirubicin (Zhong et al., 2011).

AKR1C3 has been implicated in resistance to the anthracyclines in A549 (lung adenocarcinoma), HeLa (cervical carcinoma), MCF-7 (breast cancer cells) and HCT116 (colon cancer) cells by transfection studies and restoration of drug sensitivity with the AKR1C3 inhibitor 2'-hydoxyflavanone (Hofman et al., 2014). The mechanism of AKR1C3-mediated doxorubicin resistance was examined in MCF-7/Dox resistance cells,

where overexpression of *AKR1C3* led to the loss of the tumor suppressor gene phosphatase and tensin homolog (*PTEN)* and activated protein kinase B (AKT) signaling (Zhong et al., 2015). In a microfluidic model of a stage IV glioblastoma cell line, in which the microenvironment could be manipulated, transcriptome sequencing identified overexpressed genes responsible for the early appearance of doxorubicin drug resistance, and were found to correspond to *AKR1B1, AKR1C1, AKR1C2*, and *AKR1C3* (Han et al., 2016).

Association of *AKR* overexpression with daunorubicin resistance has been observed in numerous cancer cell lines, including the overexpression of *AKR1A1* and *AKR1B1* in pancreatic adenocarcinoma cells (Plebuch, et al., 2007a); the overexpression of *AKR1B10* in lung cancer cells (Zhong et al., 2011); and the overexpression of *AKR1C3* in lung, colon, breast cancer, and cervical cancer cell lines (Hofman et al., 2014). Similarly, an association of *AKR* overexpression with doxorubicin resistance has been observed in multiple cell lines including those from cervical cancer involving *AKR1B1* (Lee et al., 2002); from nonsmall lung cancer involving *AKR1C1, AKR1C2,* and *AKR1C3* (Hung et al., 2006; Wang et al., 2007); from breast cancer involving *AKR1C2* and *AKR1C3* (Veitch et al., 2009; Heibein et al., 2012); and from gastric cancer involving *AKR1B1* (Morikawa et al., 2015). Resistance to idarubicin and epirubicin has also been linked to the expression of AKR enzymes in breast cancer with involvement from AKR1C1, AKR1C2 (Veitch et al., 2009), and AKR1C3 (Hofman et al., 2014), with involvement of AKR1C3 (Hofman et al., 2014) and AKR1B10 in lung cancer *(*Zhong et al., 2011*)*, and with involvement of AKR1C3 in colon and cervical cancer cell lines (Hofman et al., 2014). It is uncertain whether the mechanism of resistance mediated by AKRs is due to carbonyl reduction on the side chain, elimination of 4-HNE, or both. The relative contribution of AKR1C2 to doxorubicin resistance was assessed in MCF-7 cells. It was found that MCFDox-2 cells (that were ABC transporter null) could have their resistance reversed by 5β-cholanic acid (an AKR1C2 inhibitor) but, in cells that were resistant to epirubicin and overexpressed an ABC transporter, the AKR1C2 inhibitor was without effect (Veitch et al., 2009).

B. Mitomycin

Mitomycin is widely used for patients with advanced metastatic cancers of the stomach and colon. Mitomycin undergoes both enzymatic and spontaneous rearrangement to form bifunctional or trifunctional alkylating agents (Wellestein, 2018). It cross links DNA at the N6 position of adenine and the O6 and N7 position of guanine inhibiting DNA synthesis. Mitomycin is also a *p*-quinone which can redox cycle both enzymatically and nonenzymatically to the hydroquinone. The principal enzyme involved in two electron reduction of the *p*-quinone is NADPH: quinone oxidoreductase (NQO1). Formation of the hydroquinone can lead to its oxidation back to the quinone with consumption of molecular oxygen and the formation of ROS, establishing a futile redox cycle leading to oxidative damage of lipids and DNA. AKRs also have the potential to catalyze two electron quinone reduction to form the hydroquinone and exacerbate mitomycin redox cycling. However, in both instances, formation of the hydroquinone prevents the formation of the more reactive semiquinone anion radical and can lead to elimination of the hydroquinone as conjugates (Fig. 5). Thus, two mechanisms of chemoresistance can exist, formation of the hydroquinone for conjugation and elimination of lipid peroxide breakdown products resulting from ROS formation. 4-HNE is inactivated principally by AKRB10 in the colon and may contribute to mitomycin drug resistance by eliminating the cytotoxic 4-HNE (Matsunaga et al., 2011). AKR1B10 is considered a potential biomarker for colon cancer and tumors of the small intestine and was originally named as "small intestine-like" aldose reductase. Notably, both NQO1 and AKR1B10 are regulated by the NRF2-KEAP1 pathway. The contribution of AKR1B10 to mitomycin drug resistance was assessed in nonresistant HCT-15 colon cells where overexpression of AKR1B10 and silencing of AKR1B10 decreased and increased sensitivity to the drug (Matsunaga et al., 2011).

C. Cis-Platin and Platin-Based Drugs

Cis-platin is transported into cancer cells by a high affinity Cu^{2+} solute carrier transporter. Upon aquation, it loses its chloride ligands and the activated platinum complexes react with DNA and sulfhydryl groups to form protein-DNA cross links that inhibit transcription (Wellestein, 2018). Simpkins showed that growth of ovarian cancer cells in the presence of *cis*-platin made them drug-resistant to platin therapy. Gene expression analysis identified dihydrodiol dehydrogenase (*AKR1C1*) as one the most overexpressed genes in the resistant cells (Deng et al., 2002). To demonstrate causality, transfection of the AKR1C1 cDNA into parental ovarian cancer cells established the drug-resistant phenotype. Subsequent studies showed dihydrodiol dehydrogenase 1 and 2 (AKR1C1 and AKR1C2) were responsible for *cis*-platin and carboplatin resistance in human ovarian, cervical, lung, and germ cell tumor cell lines (Deng et al., 2004). Transfection of si-RNA against *AKR1C1* and *AKR1C2* in 2008/C13 cells restored sensitivity to *cis*-platin with a concomitant production of ROS (Chen et al., 2008). In the *cis*-platin-resistant HeLa cervical cell line silencing of *AKR1C1, AKR1C2, AKR1C3*, and *AKR1C4* restored drug sensitivity (Shiiba et al., 2017). AKR1B10 was later implicated in *cis*-platin resistance by eliminating the accumulation of 4-HNE formed. The resistance was also accompanied by downregulation of PPARγ, and sensitivity could be restored by the

transfection of PPARγ and the addition of a PPARγ agonist rosiglitazone (Matsunaga et al., 2016b). Thus, exposure to *cis*-platin produces both ROS and an electrophilic signal (4-HNE) to activate the NRF2-KEAP1 pathway and induce *AKR* genes. The downregulation of PPARγ is a pro-proliferative response and could counter the cytotoxic effects of *cis*-platin.

AKR overexpression has since been associated with resistance to *cis*-platin in many different cancer cells including: *AKR1B1* and *AKR1C1* in cervical cancer (HeLa) cells (Lee et al., 2002; Shiiba et al., 2017); *AKR1C1* and *AKR1C3* in colon cancer cells (Matsunaga et al., 2013); *AKR1C1* and *AKR1C2* in nonsmall cell lung carcinoma (Chen et al., 2010; Wang et al., 2007); *AKR1C1, AKR1C2, AKR1C3*, and *AKR1C4* in oral cancer (Shiiba et al., 2017); *AKR1C1* in head and neck squamous carcinomas (Chang et al., 2019); *AKR1C1* in nasopharyngeal carcinoma (Zhou et al., 2020), where loss of *AKR1C1* expression was a good prognostic indicator of chemosensitivity to *cis*-platin; and overexpression of *AKR1C1*, AKR1C2, and AKR1C3 in metastatic bladder cancer were associated with resistance to *cis*-platin (Matsumoto et al., 2016).

Association of *AKR* overexpression with resistance to carboplatin has also been seen in cancer cell lines including: *AKR1C1* and *AKR1C2* in cervical cancer (Deng et al., 2004); *AKR1C1* and *AKR1C2* in ovarian cancer (Deng et al., 2004); *AKR1C1* and *AKR1C2* in metastatic lung cancer and germ cell tumors (Deng et al., 2004); and in oxaliplatin resistance in gastric cancer involving *AKR1C1* and *AKR1C3* overexpression (Chen et al., 2013). Thus, *AKR* overexpression is part of the cancer chemotherapeutic resistance phenotype to platin-based cancer chemotherapeutics.

D. Methotrexate

Methotrexate is a potent competitive inhibitor of dihydrofolate reductase and prevents the formation of tetrahydrofolate, which is required for the de novo synthesis of thymidine and blocks DNA synthesis in rapidly proliferating cells (Rajagopalan et al., 2002). Methotrexate produces an 80%–90% cure rate in choriocarcinoma, but drug resistance ensues in the remaining patients. JEG-3 choriocarcinoma cells can be made methotrexate drug-resistant by the overexpression of *AKR1C3* and cells can be sensitized to the drug by AKR1C3 small hairpin-RNA. Increased drug sensitivity is associated with increased ROS formation, apoptosis, and cell cycle arrest (Zhao et al., 2014). Methotrexate drug resistance has since been observed in HT29 human colon cancer cells and is associated with the overexpression of *AKR1C1*. *AKR1C1* was shown to be transcriptionally regulated by phosphorylated Sp1 and Sp3 in this instance (Selga et al., 2008). It is difficult to assess the contribution of AKR1C isoforms to methotrexate resistance in JEG-3 cells since other mechanisms contribute, which include the overexpression of the chloride intracellular channel protein-1 and multi-drug resistance-associated protein (MRP1) (Wu et al., 2017).

E. Oracin

Oracin is a promising cytostatic drug which is inactivated by carbonyl reduction by stereoselective reduction of its 11-keto group (Fig. 6). The enzyme most implicated in this transformation is AKR1C1 when AKRC1, AKR1C2, and AKR1C4 were compared, where AKR1C1 gave predominately the (+) enantiomer (Wsol et al., 2007). Subsequent studies showed that AKR1C3 was also involved (Novotna et al., 2008). Since the *ARK1C* genes are upregulated by stress response, it is not surprising that their overexpression may lead to drug resistance resulting in increased metabolism of oracin. The relative contribution of AKRs to oracin drug resistance has yet to be determined.

F. Vincristine

Vincristine is a vinca alkaloid and an antitubulin agent that prevents the proliferation of rapidly growing cells by preventing formation of the mitotic spindle (Jordan, 2002). Vincristine is the drug of choice for T-cell acute lymphoblastic leukemia (T-ALL). T-ALL cells from therapy-resistant patients overexpress *AKR1C1-AKR1C3*, and decreased expression using si-RNA improved sensitivity to the drug (Bortolozzi et al., 2018). Furthermore, categorization of patients as therapy responders or nonresponders based on minimal residual disease showed that minimal residual disease–positive resistant patients had high levels of AKR1C1, AKR1C2, and AKR1C3. As well as acting as an antitubulin, vinca alkaloids can cause collapse of the mitochondria, elevating ROS production. As the expression of AKR1C isoforms is a component of the antioxidant response in humans, it is not surprising that their upregulation may attenuate the effect of the vinca alkaloids. Other antitubulin-based agents, e.g., taxanes, can upregulate drug efflux pumps, e.g., ATP-Binding

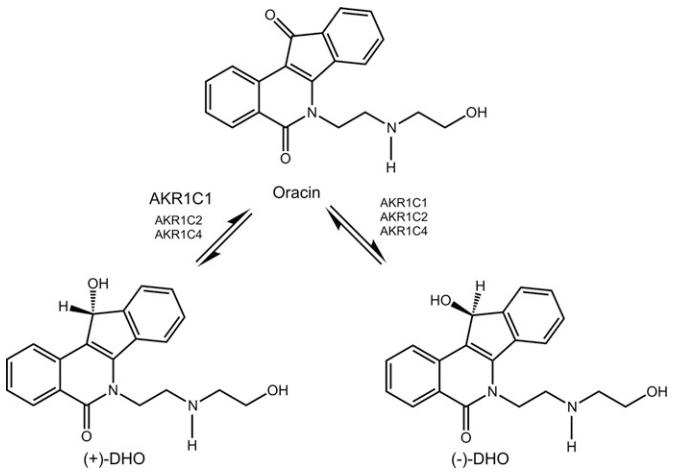

Fig. 6. Inactivation of oracin by carbonyl reduction by AKRs.

Cassette Subfamily B transporters, indicating that different drug-resistant mechanisms may work concurrently to prevent a chemotherapeutic drug response (Matsunaga et al., 2016a).

G. Irinotecan

Irinotecan is a topoisomerase inhibitor often used to treat colon cancer and small lung cell cancer in combination with fluorouracil or *cis*-platin, respectively. Two dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by proteomic analysis by in-gel digest with trypsin followed by electrospray ionization-quadrupole time-of-flight mass spectrometry identified 15 proteins that were overexpressed in irinotecan-resistant LoVo (human colon adenocarcinoma) cells versus the parental cells. One of these proteins was assigned as AKR1A1 based on coverage obtained with 12 tryptic peptides (Peng et al., 2010). However, the exact role of AKR1A1 in Irinotecan drug resistance remains to be determined. Recently, Irinotecan resistance was linked to AKR1C3 upregulation. Matsunaga et al. (2020) observed increased AKR1C3 expression and activity in Irinotecan resistant colon cancer cell lines DLD-1, RKO, and LoVo as compared with parental sensitive cells. In these cells, AKR1C3 overexpression decreased and AKR1C3 silencing restored chemosensitivity. Treatment of parental colon cancer cells with Irinotecan leads to formation of cytotoxic 4-HNE. In chemoresistant cells, higher 4-HNE reductase activity and consequently lower 4-HNE levels were seen as compared with parental cells. As AKR1C3 is one of several AKR1C enzymes that can inactivate 4-HNE (Burczynski et al., 2001), the authors concluded that 4-HNE detoxification by AKR1C3 may represent the major resistance mechanism to Irinotecan.

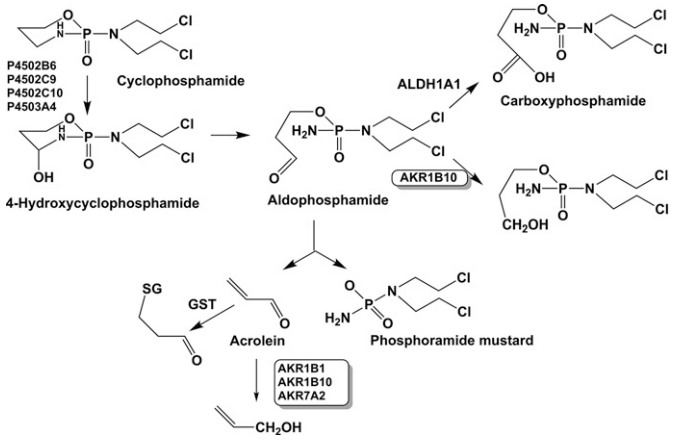

Fig. 7. Competing roles of ALDH1A1 and AKRs in the metabolism of cyclophosphamide.

H. Cyclophosphamide

Cyclophosphamide is activated by P450 isoforms (2A6, 2C9, 2C10, and 3A4) to the active metabolite 4-hydroxycyclophosphamide, which exists in its tautomeric form aldophosphamide, and this aldehyde is detoxified by aldehyde dehydrogenase (ALDH) to yield carboxyphosphamide. A portion of aldophosphamide can decompose to yield phosphoramide mustard and acrolein, which are the cytotoxic species responsible for the ability of cyclophosphamide to inhibit the growth of rapidly proliferating cells (Fig. 7). Examination of gene expression profiles in medulloblastoma cell lines resistant to cyclophosphamide showed a 20-fold elevation in *AKR1B10* expression. AKR1B10 may work in concert with ALDH1A1 to inactivate the reactive aldehyde aldophosphamide and contribute to cyclophosphamide resistance (Bacolod et al., 2008). Acrolein can also be eliminated by AKR1B1, AKR1B10, and AKR7A2, where AKR1B10 is the major contributor (Shen et al., 2011; Li et al., 2012a) However, because acrolein is a strong electrophile, its major route of metabolism will be GSH conjugation. The elimination of the aldophosphamide by AKR1B10 is likely a major contributing mechanism of cyclophosphamide drug resistance.

I. Tyrosine Kinase Inhibitors

Several tyrosine kinase inhibitors such as imatinib, nilotinib, erlotinib, and ponatinib are currently used for the treatment of some cancers. The AKRs do not appear to be involved in resistance to these agents. To the contrary, the tyrosine kinase inhibitor ibrutinib is an inhibitor of AKR1C3 and can counteract anthracycline drug resistance mediated by AKR1C3 (Morell et al., 2020). Apatinib also inhibits the NRF2 pathway which would lead to a downregulation of *AKR* genes regulated by NRF2 and could surmount drug resistance to other chemotherapeutic agents (Sun et al., 2020). Imatinib, nilotinib, and ponatinib are amides, while erlotinib does not contain a carbonyl group and therefore, these compounds would not be substrates for AKRs.

V. Resistance to Antihormonal Therapies Mediated by Aldo-Keto Reductases

AKR1C enzymes are intimately involved in the biosynthesis and metabolism of steroid hormones. In particular, AKR1C3 is one of the principal enzymes involved in peripheral androgen biosynthesis and is upregulated by androgen deprivation in castration-resistant prostate cancer, the lethal form of prostate cancer (Hofland et al., 2010; Hamid et al., 2013; Mitsiades et al., 2012). As a result, AKR1C3 is implicated in drug resistance to the new androgen receptor signaling inhibitors (ARSI), e.g., abiraterone acetate (an inhibitor of P450c17: 17α-hydroxylase/17,20-lyase)

and enzalutamide (a potent androgen receptor antagonist) used in the treatment of castration-resistant prostate cancer (Scher et al., 2010; Fizazi et al., 2012).

The 17-ketosteroid reductase activity of AKR1C3 converts 4-androstene-3,17-dione to testosterone, which can also act as substrate for the peripheral formation of 17β-estradiol via aromatase. By providing a substrate for aromatase, overexpression of *AKR1C3* may also play a role in hormonal-dependent breast cancer (Byrns et al., 2010; McNamara and Sasano, 2019). Concurrent overexpression of *AKR1C1*, which is the principal 20-ketosteroid reductase involved in the elimination of progesterone as 20α-hydroxyprogesterone, can work in concert with AKR1C3 to increase the ratio of 17β-estradiol:progesterone in estrogen-dependent malignancies (Penning et al., 2000; Rizner et al., 2006).

A. Antiandrogenic Therapy

C4-2B prostate cancer cells grown in the presence of either enzalutamide or abiraterone acquire drug resistance to these agents and overexpress AKR1C3 (Liu et al., 2015; Liu et al., 2017). Xenografts of these resistant cells fail to respond to these drugs, but indomethacin (INDO), a potent and selective inhibitor of AKR1C3, can surmount this drug resistance and have antitumor activity. AKR1C3 is repressed by androgens and upregulated by androgen deprivation (Hofland et al., 2010; Hamid et al., 2013; Mitsiades et al., 2012). Enzalutamide is a potent androgen receptor (AR) antagonist and can remove the repressive effects of the AR on the *AKR1C3* promoter leading to overexpression of *AKR1C3*. This repressive effect can also be surmounted by overexpression of the TMPRSS2-ERG, a fusion protein that arises in late-stage prostate cancer. Erythroblast transformation specific related gene replaces the AR on the *AKR1C3* promoter, and the resultant increase in AKR1C3 in concert with steroid 5α-reductase leads to more 5α-dihydrotestosterone to bind to AR (Powell et al., 2015). This leads to a feed-forward mechanism to increase *TMPRSS2-ERG* expression and maintain AKR1C3 levels to sustain ARSI drug resistance (Powell et al., 2015).

Abiraterone may also deprive the AR of its ligand leading to removal of the repressive effects of AR on the *AKR1C3* promoter. This could be accomplished by inhibition of P450c17 in both the adrenal and the tumor itself. However, evidence for de novo synthesis of C19 steroids in prostate tissue is poor, and instead, the mechanism may involve inhibition of 3β-hydroxysteroid dehydrogenase type 1 by the delta-4-abiraterone metabolite (Li et al., 2012b).

B. Antiestrogenic Therapy

Exemestane is a steroidal based aromatase inhibitor used in the endocrine therapy of hormonal-dependent breast cancer. Its primary metabolite is 17β-dihydroexemestane, which is produced by carbonyl metabolism (Platt et al., 2016). While 17β-dihydroexemestane can still function as an aromatase inhibitor, it can also undergo glucuronidation by UDP-glucuronosyltransferases leading to its elimination. Carbonyl reductases responsible for the formation of 17β-dihydroexemestane include the AKR1C enzymes (Platt et al., 2016). However, AKR1C3 has very low catalytic efficiency for exemestane making it doubtful that it is a major mechanism of drug resistance.

VI. Inactivators of the Nuclear Factor-Erythroid 2 p45-Related Factor 2-Kelch-Like ECH-Associated Protein 1 System

The preceding sections make the case that NRF2 inactivators would sensitize tumor cells to chemotherapy. Natural products exist that act as NRF2-inactivators that would counter this effect, e.g., the quassinoid brusatol, luteolin, chrysin, and wogonin. Brusatol was identified as a natural product inhibitor of NRF2, but it does not bind to the transcription factor directly. Rather, it is an inhibitor of both cap-dependent and cap-independent translation, which is required for the stability of short-lived proteins such as NRF2 (Harder et al., 2017). By contrast, flavonoids such as chrysin repress NRF2 expression through downregulation of the P13K-AKT and extracellular signal-regulated kinase (ERK) pathway (Gao et al., 2013). High-throughput screening using ARE-luciferase reporters gene constructs could be used to identify compounds that would attenuate NRF2 signaling and act as NRF2 inactivators.

VII. Inhibitors of Aldo-Keto Reductases to Surmount Drug Resistance

The association of AKR forced overexpression with chemotherapeutic drug resistance, *AKR* overexpression in cancer cell lines, and genetic silencing of *AKRs* to reverse drug resistance suggests that AKR inhibitors could be exploited to surmount drug resistance. A number of AKR inhibitors have been used in vitro and in vivo for this indication and are summarized in Table 3 and (Fig. 8). However, it is apparent that for many chemotherapeutic agents, a battery of AKR enzymes may be involved, suggesting that if only one AKR is inhibited, others may substitute. Thus, both AKR isoform-selective inhibitors have been used as well as pan-AKR1C inhibitors. One major class of drugs that inhibits the AKR1C isoforms are the nonsteroidal anti-inflammatory drugs (NSAIDs) which can discriminate between the four AKR1C isoforms (Byrns et al., 2008). For example, salicylate is an AKR1C1 isoform-selective inhibitor, and INDO is an AKR1C3 isoform-selective inhibitor, whereas flufenamic acid is a pan-AKR1C inhibitor (Byrns et al., 2008; El-Kabbani et al., 2010; El-Kabbani et al., 2011). NSAIDs and their

TABLE 3
Drug resistance to chemotherapeutic agents reversed by AKR inhibitors

AKR	AKR Inhibitors	Chemotherapeutic Drug Resistance Inhibited	Cancer/Cell Line	Reference
Platinum-Based Drugs				
AKR1B1	1-Benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate (EBPC)	*Cis*-platin	Cervical cancer HeLa	(Lee et al., 2002)
AKR1C1, AKR1C2, AKR1C3, AKR1C4	Mefenamic acid		+ mouse xenograft	(Shiiba et al., 2017)
AKR1C1 AKR1C3	3-Bromo-5-phenylsalicylic acid (AKR1C1) Tolfenamic acid (AKR1C3)		Colon cancer HCT15	(Matsunaga et al., 2013)
AKR1C1, AKR1C2, AKR1C3, AKR1C4	Mefenamic acid		Oral squamous cell carcinoma Sa3	(Shiiba et al., 2017)
AKR1C1	3-Bromo-5-phenylsalicylic acid (Ki = 4 nM)		Head and neck squamous carcinoma 293 T, FaDu Cal-27, HSC-2 and HSC-4	(Chang et al., 2019)
AKR1C1	Flufenamic acid		Metastatic bladder cancer UM-UC-3	(Matsunaga et al., 2016a)
AKR1C1, AKR1C2, AKR1C3	Phenolphthalein α-methylcinnamic acid, Flufenamic acid	Oxaliplatin	Gastro-intestinal tract cancer TSGH	(Chen et al., 2013)
Anthracyclines				
AKR1B1	1-Benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate	Doxorubicin	Cervical cancer HeLa	(Lee et al., 2002)
AKR1C2	5β-Cholanic acid-3α,7α-diol		Breast cancer MCF-7	(Veitch et al., 2009)
AKR1B10, AKR1C3, AKR1C2	5β-Cholanic acid-3α,7α-diol		Breast cancer MCF-7	(Heibein et al., 2012)
AKR1B10	Oleanoic acid 3-(4-hydroxy-2-methoxyphenyl-1)acrylic acid 3-(3-hydroxyphenyl)propyl ester		Gastric cancer MKN45	(Morikawa et al., 2015)
AKR1C1 AKR1C3	3-Bromo-5-phenylsalicylic acid, Tolfenamic acid,	Daunorubicin	Leukemia (acute monocytic leukemia) U937	(Matsunaga et al., 2014)
AKR1B10 AKR1C3	Emodin		Lung cancer A-549 Liver cancer Hep-G2	(Hintzpeter et al., 2016)
AKR1C3	Purvalanol A Roscovine		Colon cancer HCT 116	(Novotná et al., 2018a)
	Dinaciclib			(Novotná et al., 2018a, 2018b)
AKR1C3	Irutinib		Colon cancer	(Morrell et al., 2020)
	Acalabrutinib		HCT 116	(Bukum et al. 2019)
	Buparlisib			
AKR1B10	Epalrestat	Daunorubicin, Idarubicin	Lung cancer NCI-H460	(Zhong et al., 2011)
AKR1C3	2-Hydroxyflavanone		Lung cancer A-549	(Hofman et al., 2014)
Other drugs				
AKR1B10	(Z)-2-(4-methoxyphenyl-imino)-7-hydroxy-N-(pyridin-2-yl)-»H-chromene-3-carbox-amide	Mitomycin-c	Colon cancer HT-29	(Matsunaga et al., 2011)
AKR1C3	Baccharin derivatives	Etoposide	Leukemia (acute myeloid leukemia) HL-60, KG-1a	(Verma et al., 2016)

(*continued*)

TABLE 3— *Continued*

AKR	AKR Inhibitors	Chemotherapeutic Drug Resistance Inhibited	Cancer/Cell Line	Reference
		Platinum-Based Drugs		
AKR1C1, AKR1C2, AKR1C3	6-Medroxy-progesterone acetate	Vincristine	Leukemia (acute lymphoblastic leukemia) CCRF-CEM, DND-41, Loucy	(Bortolozzi et al., 2018)
AKR1C1, AKR1C2, AKR1C3, AKR1C4	Mefenamic acid	5-Fluoro uracil	Cervical cancer HeLa + mouse xenograft	(Shiiba et al., 2017)
AKR1C3	1,3,5-Trisubstituted amido cinnamic acids Compounds Kv-49a, Kv-49g	Cytarabine	Leukemia (acute myeloid leukemia) HL-60, TMP-1, KG-1a (T-cell acute lymphoblastic leukemia) primary cells	(Verma et al., 2019)
AKR1C3	Tolfenamic acid	Irinotecan	Colon cancer cell lines DLD1, RKO, LoVo	(Matsunaga et al., 2020)
		Antihormone Therapy		
AKR1C3	Indomethacin	Enzalutamide Abiraterone Acetate	Castration resistant prostate cancer	(Liu et al., 2015; 2017)
AKR1C3	Kv-37 and related Baccharin analogs	Enzalutamide	Castration resistant prostate cancer	(Verma et al., 2018)
	1,3,5-Trisubstituted amido cinnamic acids Compound Kv-49g	Apalutamide Darolutamide	Castration resistant prostate cancer	(Morsy & Trippier, 2020)

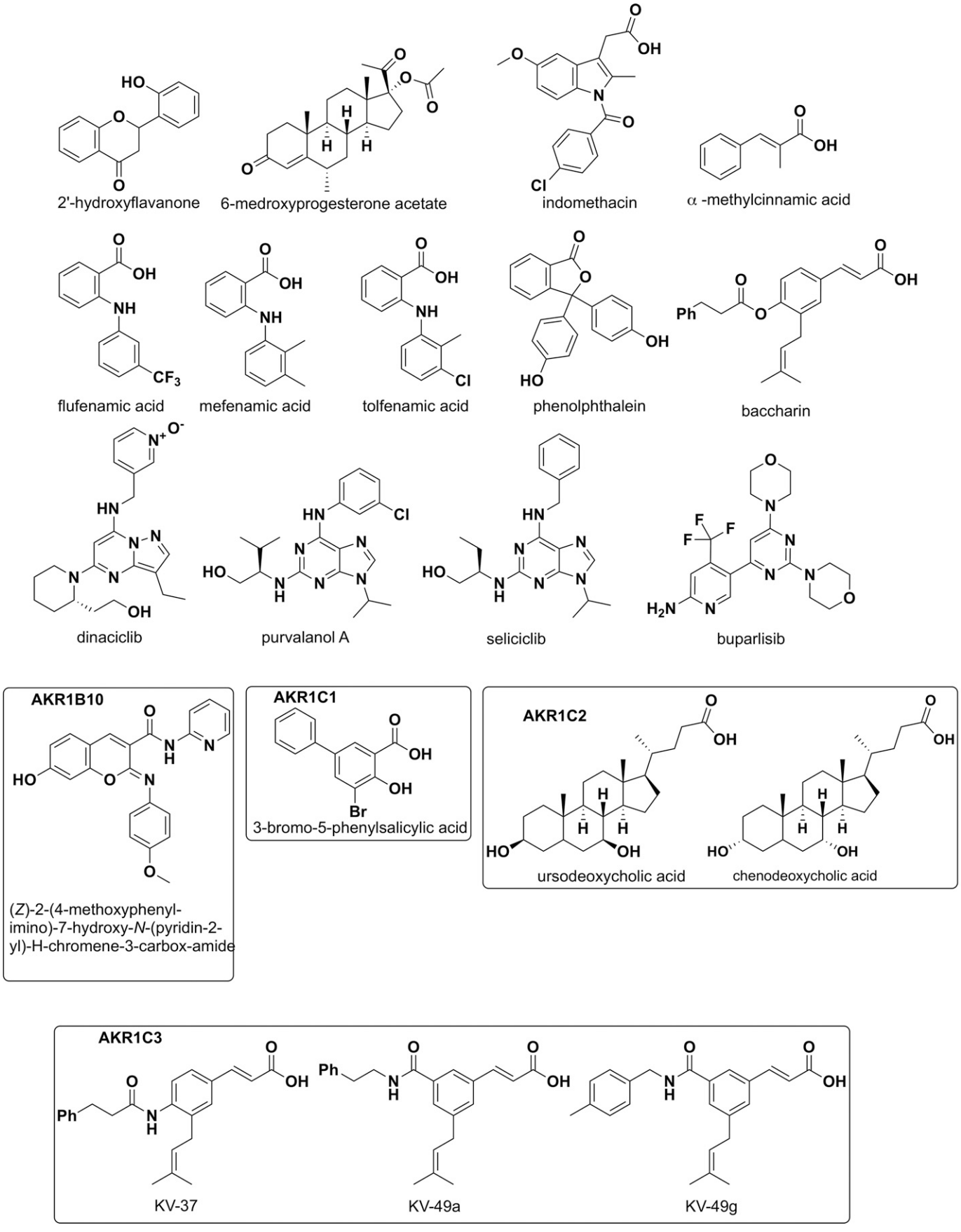

Fig. 8. Structures of common AKR inhibitors including KV-37 and the Baccharin analogs.

analogs have played a prominent role in determining the utility of AKR inhibitors to surmount drug resistance, especially in the case of *cis*-platin.

A. Anthracycline Chemotherapeutics and Aldo-Keto Reductase Inhibitors

AKR inhibitors have been tested in vitro to restore sensitivity to anthracycline drugs in lung, breast, gastric, liver, and colon cancer cell lines and in leukemia (Veitch et al., 2009; Zhong et al., 2011; Heibein et al., 2012; Matsunaga et al., 2013; Hofman et al., 2014; Matsunaga et al., 2014; Morikawa et al., 2015; Hintzpeter et al., 2016; Novotná et al., 2018a, 2018b; Bukum et al., 2019). In the lung adenocarcinoma A549 cell line which has high endogenous expression of AKR1C3, 10 μM 2-hydroxyflavanone, a specific AKR1C3 inhibitor, restored cytotoxicity to daunorubicin and idarubicin (Hofman et al., 2014). In addition, 30 μM emodin, a potent inhibitor of AKR1B10 and AKR1C3, decreased viability of these cells when treated with 0.5 and 1 μM daunorubicin (Hintzpeter et al., 2016). Treatment with 100 μM epalrestat, an AKR1B10 inhibitor, decreased resistance to daunorubicin and idarubicin in the lung cancer NCI-H460 cell line, which has high expression of *AKR1B10* but no expression of *AKR1B1* (Zhong et al., 2011).

In MCF-7 breast cancer cells made resistant to doxorubicin, high levels of *AKR1B10, AKR1C3*, and *AKR1C2* were expressed, and the specific AKR1C2 inhibitor 5β-cholanic acid-3α,7α-diol restored chemo-sensitivity and prevented nuclear localization of doxorubicin, which is crucial for its action as a topoisomerase II inhibitor. This effect was not seen in epirubicin-resistant or control MCF-7 cells (Veitch et al., 2009; Heibein et al., 2012).

In the MKN45 gastric cancer cell line, Morikawa et al. (2015) observed that exposure to doxorubicin upregulates expression of *AKR1B10*, *AKR1C1*, *AKR1C2*, and *AKR1C3*, but only the AKR1B10 inhibitors; 20 μM oleanoic acid or 10 μM 3-(4-hydroxy-2-methoxyphenyl-1)acrylic acid 3-(3-hydroxyphenyl)propyl ester significantly sensitized these cells to 60 μM doxorubicin. By contrast, the specific AKR1C1 (3-bromo-5-phenylsalicylic acid), AKR1C2 (ursodeoxycholic acid), and AKR1C3 [*E*-3-(3-(3-hydroxybenzyl)oxy)-4-(3-phenylpropanoyl)oxy)-phenyl)acrylic acid] inhibitors had no effect on cell viability. These results demonstrate that AKR1 isoform specific inhibitors could be used to determine their individual contributions to the drug resistance phenotype.

In the colon carcinoma HCT116 cell line transiently transfected with AKR1C3, the purine based analog cyclin-dependent kinase inhibitors, roscovine and purvalanol A, potently inhibited the metabolism of clinically relevant concentrations of 1.0 μM daunorubicin, yielding IC_{50} values of 3.7 μM for roscovine and an IC_{50} value of 6.4 μM for purvalanol A. In the same cells, the cyclin dependent kinase inhibitor dinaciclib inhibited 1 μM daunorubicin metabolism and acted as a potent inhibitor of AKR1C3 and a modest inhibitor of AKR1B10 yielding IC_{50} values of 0.22 μM and 181 μM respectively for these enzymes. Dinaciclib was found to be a tight-binding inhibitor of AKR1C3 with a K_i 0.07 μM, and sensitivity to daunorubicin was restored when HCT116-AKR1C3 cells were preincubated with dinaciclib (0.1 μM) for 2 hours and then treated with 0.1 to 1.0 mM daunorubicin (Novotná et al., 2018a, b), The 2-hour pretreatment of cells with dinaciclib mimics the 2-hour intravenous infusion used to administer the drug in clinical trial (Flynn et al., 2015).

Recently, a pan-phosphoinositide-3-kinase inhibitor, buparlisib, which is under clinical evaluation for treatment of different cancers, was reported to inhibit AKR1C3 with a K_i = 14 μM. In colon cancer HCT116-AKR1C3 cells, buparlisib inhibited the reduction of 1 μM daunorubicin with an IC_{50} value = 7.9 μM (Bukum et al., 2019). In human leukemic U937 cells, daunorubicin induced expression of *AKR1C1* and *AKR1C3* and decreased cell sensitivity to the drug and 4-HNE, while pretreatment with 20 μM 3-bromo-5-phenylsalicylic acid, an AKR1C1 inhibitor, or 20 μM tolfenamic acid, an AKR1C3 inhibitor, restored sensitivity to daunorubicin. The specific AKR1C2 inhibitor ursodeoxycholic acid had no effect on cell viability or drug sensitivity (Matsunaga et al., 2014).

In summary, human enzymes of the AKR1B and AKR1C family mediate resistance to the anthracyclines, and inhibitors of these enzymes can restore drug sensitivity. However, the AKRs involved can vary by cancer cell line depending on their expression level and differing substrate specificity for the anthracycline family of therapeutics, suggesting that precision therapy with these inhibitors would require tumor biopsies for RNA analysis or protein expression.

B. Cis-platin Therapeutics and Aldo-Keto Reductase Inhibitors

The *N*-phenylanthraniliates, flufenamic acid, and mefenamic acid which are NSAIDs restore sensitivity to *cis*-platin (Morikawa et al., 2015; Shiiba et al., 2017). Specific or pan-AKR1C inhibitors have been used in colon, cervical, oral squamous, and bladder cancer cell lines to restore sensitivity to *cis*-platin, where the pan-AKR1C inhibitors flufenamic acid and mefenamic acid also showed effects in explant mouse models (Shiiba et al., 2017; Chang et al., 2019). In the *cis*-platin-resistant colon cancer cell line HCT15, expression of *AKR1C1* and *AKR1C3* increased with development of resistance. A 2-hour pretreatment with 40 μM 3-bromo-5-phenylsalicylic acid (a specific AKR1C1 inhibitor) and tolfenamic acid (a specific AKR1C3 inhibitor) or their combination increased cell susceptibility to *cis*-platin. Use of the selective AKR1C2 inhibitor ursodeoxycholic acid revealed no effect, suggesting that either a pan-AKR1C inhibitor or multiple isoform-selective inhibitors are needed. Sensitivity to *cis*-platin was enhanced when AKR1C1 and

AKR1C3 inhibitors were combined with 0.2 μM proteasomal inhibitor MG132, where a compensatory overexpression of AKR1C1 and AKR1C3 and proteasome subunits was observed (Matsunaga et al., 2013).

In the *cis*-platin–resistant HeLa cervical cell line and the oral squamous cell carcinoma cell line Sa3, which express high levels of *AKR1C1, AKR1C2, AKR1C3* and *AKR1C4*, the use of 200 and 400 μM of the pan-AKR1C inhibitor mefenamic acid restored chemosensitivity, which was confirmed in vivo after implantation of HeLa resistant cells into nude mice and administration of mefenamic acid (Shiiba et al., 2017).

In the bladder cancer cell lines UM-UC-3, 5637 and J82, treatment with *cis*-platin increased expression of *AKR1C1, AKR1C2*, and *AKR1C3*. The liver and bone metastatic cells originating from the UM-UC-3 xenograft mouse model showed further upregulation of *AKR1C1* as well as *AKR1C2* and *AKR1C3,* where *AKR1C1* was upregulated by inflammatory cytokines (IL1β and IL-6). The use of the AKR1C inhibitor flufenamic acid (10 μM) decreased UM-UC-3 cell invasion as measured by a Matrigel assay. Combined treatment with 5 μM *cis*-platin, which is commonly used for treatment of advanced bladder cancer, and 10 μM flufenamic acid restored sensitivity to *cis*-platin in liver and bone metastatic cancer cells (Matsumoto et al., 2016).

In head and neck squamous carcinoma, *AKR1C1* expression is associated with *cis*-platin resistance, where patients with higher *AKR1C1* expression have shorter median survival and recur earlier than those with lower *AKR1C1* levels (Chang et al., 2019). In the model cell lines FaDu and HSC-2 with high *AKR1C1* expression, silencing of *AKR1C1* increased chemosensitivity, but cotreatment with 500 nM 3-bromo-5-phenylsalicilic acid, a potent selective AKR1C1 inhibitor (K_i = 4 nM), showed no effects on the viability of these cells at IC_{50} concentrations of *cis*-platin, thus, the authors concluded that AKR1C1 may promote *cis*-platin resistance in an enzyme-independent manner (Chang et al., 2019).

AKR inhibitors have also been examined to alleviate resistance to oxaliplatin. In the resistant human gastric carcinoma cell line TSGH-S3, which has high expression of *AKR1C1* and *AKR1C3,* use of specific and pan-AKR1C inhibitors [phenolphthalein (0.5 – 5 μM), α-methylcinnamic acid (0.5 -5 μM), and flufenamic acid (10 -50 μM)] restored oxaliplatin resistance. Further knock-down of *NFE2L2* decreased the expression of *AKR1C1*, *AKR1C2*, and *AKR1C3* and reversed the resistance, supporting the crucial role of NRF2 in regulating drug resistance by induction of *AKR1C1-AKR1C3* (Zhou et al., 2020).

C. Other Cancer Chemotherapeutics and Aldo-Keto Reductase Inhibitors

AKR inhibitors have also been used to reverse drug resistance to other chemotherapeutic agents. In the colon cancer cell line HT29, which was resistant to mitomycin-c, *AKR1B10* was upregulated. High levels of AKR1B10 decreased production of 4-HNE and a 2-hour pretreatment with 5 μM (*Z*)-2-(4-methoxyphenyl-imino)-7-hydroxy-*N*-(pyridin-2-yl)-H-chromene-3-carbox-amide, a potent AKR1B10 inhibitor with a K_i = 2.7 nM, restored sensitivity to mitomycin-C (Matsunaga et al., 2011).

In the *cis*-platin–resistant HeLa cervical cell line, sensitivity could be restored to 5-fluoruracil by 200–400 μM mefenamic acid, a pan-AKR1C inhibitor. Chemosensitivity to 5-fluoruracil was also restored in implants in athymic mice using this strategy (Selga et al., 2008). This represents an example whereby expression of *AKR1C* can lead to a multidrug resistance phenotype to 5-fluoruracil and *cis*-platin which can be surmounted by pan-AKR1C inhibition.

Sensitization of T-ALL cells to vincristine could also be achieved by the pan-AKR1C inhibitor 6-medroxyprogesterone acetate. In addition, activation of NRF2 by *tert*-butyl hydroquinone also increased vincristine resistance (Bortolozzi et al., 2018).

In summary, specific AKR1 and pan AKR1C inhibitors can restore chemosensitivity in cell lines and explant models. Specific AKR1A1, AKR1B10, and AKR1C3 inhibitors can alleviate resistance to individual anthracyclines. AKR1C1 and AKR1C2 inhibitors or pan-AKR1C inhibitors may have roles in alleviating resistance to platinum-based chemotherapeutics. Pan-AKR1C and specific AKR1C inhibitors may also help restore sensitivity to other chemotherapeutics. The translation of these findings to the clinical setting could involve the use of existing drugs, e.g., NSAIDs to chemo-sensitize patients, whereas other isoform specific AKR inhibitors may require further preclinical development.

VIII. Inhibitors of Aldo-Keto Reductases to Potentiate the Effects of Chemotherapeutic Agents

The previous section describes the use of AKR inhibitors to sensitize cells to cancer chemotherapeutic agents. This raises the issue of whether combinations of AKR inhibitors and cancer therapeutic agents will exert a greater effect than either drug alone leading to synergistic effects to inhibit cancer cell growth. Baccharin analogs have been used to target AKR1C3 to obtain synergistic drug responses to enzalutamide, darolutamide, and apalutamide in prostate cancer cells (Verma et al., 2018; Morsy and Trippier, 2020), to etoposide and daunorubicin in AML (Verma et al., 2016), and to daunorubicin and cytarabine in AML and T-ALL (Verma et al., 2019). Synergism was measured using the Chou-Talalay median-effect plot (Chou and Talalay, 1984; Chou, 2010), and in many cases synergism exceeded 200-fold. The observed synergism can be accounted for by the mode-of-action of AKR1C3 in these diseases. In prostate cancer AKR1C3 synthesizes potent androgens for the AR

which can bypass the therapeutic efficacy of ARSIs (Adeniji et al., 2013; Knudsen and Penning, 2010). In leukemia, the prostaglandin F synthase activity of AKR1C3 prevents signaling via PPARγ and promotes cellular proliferation.

A. Prostate Cancer

The cinnamic acid derivative KV-37 is a highly selective AKR1C3 inhibitor that has 109-fold selectivity over the AKR1C2 isoform with an IC_{50} of 66 nM for AKR1C3 inhibition (Fig. 8). KV-37 was found to exert a high degree of synergistic drug interaction upon combination with enzalutamide that results in >200-fold potentiation of cytotoxicity. In the enzalutamide-resistant, high AKR1C3–expressing 22Rv1 prostate cancer cell line (Verma et al., 2018), pretreatment of 22Rv1 cells with KV-37 followed by exposure to enzalutamide, when stimulated to grow in the presence of the AKR1C3 substrate (4-androstene-3,17-dione), resulted in a 208-fold potentiation effect to provide a combination IC_{50} of 4.3 μM for enzalutamide and KV-37, which is well below the maximum achievable plasma concentration of enzalutamide in patients. Further evidence that AKR1C3 is responsible for enzalutamide resistance was demonstrated in LNCaP cells stably transfected to overexpress AKR1C3 (LNCaP1C3), wherein the cytotoxicity of enzalutamide was significantly reduced (IC_{50} = 150 μM) compared with AKR1C3-null LNCaP cells (IC_{50} = 50 μM). Enzalutamide resistance was again reversed upon pretreatment with KV-37 (yielding a combination IC_{50} = 8.19 μM). The higher combination IC_{50} values seen in the LNCaP1C3 cells over the 22Rv1 cells is due to the higher expression of AKR1C3 in the LNCaP1C3 cell line. The AKR1C3 inhibitor alone and in combination with enzalutamide resulted in increased apoptosis, and attenuated AR transactivation and PSA expression in both the 22Rv1 and LNCaP1C3 cells.

A second generation AKR1C3 inhibitor developed around the same chemical scaffold, KV-49g, was >2800-fold more selective for AKR1C3 over AKR1C1 and AKR1C2, possessed an IC_{50} of 70 nM, and was found to potentiate the effects of two newly approved AR antagonists apalutamide (ARN) and darolutamide (ODM) (Morsy and Trippier, 2020). Upon 24-hour pretreatment of cell lines with KV-49g, a potentiation effect of 126-fold and 75-fold was observed with ARN and ODM, respectively.

INDO acts as an AKR1C3 inhibitor (IC_{50} = 100 nM, with an AKR1C3 selectivity = 365-fold). Its clinically approved status positions the compound to bring about immediate benefits to patients with prostate cancer (Liu et al., 2015) but is likely to lead to gastrointestinal side effects because of its COX-1 inhibition activity. In in vitro model systems, INDO exhibited lower potentiation effects in combination with enzalutamide than the baccharin analogs. In LNCaP1C3 cells, INDO conferred a 6.4-fold potentiation effect affording a combination IC_{50} = 31.4 μM compared with KV-37 (25-fold potentiation and combination IC_{50} = 8.19 μM). Thus, more potent AKR1C3 inhibitors confer a greater potentiation effect. This was further confirmed in potentiation studies of ARN and ODM in 22Rv1 cells in which a combination of INDO provided an 8-fold potentiation effect yielding a combination IC_{50} = 30 μM, whereas KV-49g produced a 126-fold potentiation effect with a combination IC_{50} = 12.47 μM for ARN and 75-fold potentiation effect with a combination IC_{50} = 5.64 μM for ODM. Since inhibition of AKR1C3 by INDO has been shown to overcome resistance to enzalutamide and abiraterone acetate both in vitro and in vivo, with the combination resulting in a significant reduction in tumor volume over three weeks in CWR22Rv1 xenografts using just 3 mg/kg i.p. of INDO (Liu et al., 2015), the KV-compounds are expected to provide superior efficacy.

The ability of INDO to potentiate abiraterone acetate and enzalutamide has led to the search for more potent AKR1C3 inhibitors based around its chemical scaffold. In 2013, Liedtke et al. (2013) developed INDO analogs, wherein derivatives exhibited nanomolar potency, with >100-fold selectivity for AKR1C3 over the AKR1C1 and AKR1C2 isoforms. These compounds also inhibited the formation of testosterone in the LNCaP1C3 cell line (Liedtke et al., 2013). However, studies have yet to be conducted to determine their ability to potentiate the effect of AR antagonists.

Even though flufenamic acid is a potent AKR1C3 inhibitor, it causes COX-2–dependent target effects. To circumvent this drawback, a series of N-phenylanthranilic acid derivatives were synthesized that retain nanomolar potency for AKR1C3 and show >100-fold selectivity but no longer inhibit COX-1 or COX-2 (Adeniji et al. 2012, 2013). Like the INDO analogs, these have not been tested for synergistic effects with either enzalutamide or abiraterone acetate. Pippione et al. (2017) replaced the benzoic acid moiety in flufenamic acid to generate hydroxytriazole derivatives with potent and selective AKR1C3 inhibition activity but without COX-2 effects. These derivatives were evaluated for antiproliferative activity in the abiraterone acetate and enzalutamide resistant AKR1C3 expressing 22Rv1 cell line and exhibited moderate antiproliferative activity with IC_{50} values of 67 and 81 μM, respectively. When one lead compound, or abiraterone acetate and enzalutamide, were used as single agents, cell viability was inhibited by approximately 20%–30%, whereas in combination a synergistic effect on cell viability was observed (Pippione et al., 2017). When the benzoic acid moiety of flufenamic acid was replaced with an acidic hydroxybenzoisoxazole moiety, a new lead compound, possessing an IC_{50} of 27 μM, was identified which showed approximately 450-fold selectivity for AKR1C3

compared with the AKR1C2 isoform, with minimum COX mediated off-target effects. When 22RV1 cells were exposed for 72 hours to 20 μM lead compound in combination with either abiraterone or enzalutamide, cell viability was decreased from 30% with the compound alone and to 50% for the drug combination (Pippione et al., 2018).

B. Leukemia

AKR1C3 is known to catalyze the synthesis of prostaglandins that sustain growth of myeloid precursors in the bone marrow. The enzyme is overexpressed in several subtypes of AML and in T-ALL. This overexpression results in chemoresistance toward etoposide (Verma et al., 2016), the anthracyclines, and cytarabine (Verma et al., 2019).

The cotreatment of selective AKR1C3 inhibitors KV-49a and KV-49g with daunorubicin did not result in an appreciable potentiation effect in the AML cell lines HL-60, THP-1, and KG1a, which have low, moderate, and high *AKR1C3* expression, respectively. However, upon 24-hour pretreatment with either compound, potentiation of the activity of daunorubicin was observed, with >100-fold potentiation effect in the daunorubicin-resistant cell line KG1a. In the THP-1 cell line, a 16-fold potentiation effect was noted while in the HL-60 cell line and the effect of daunorubicin was potentiated by 1.3–1.7-fold. A direct correlation between *AKR1C3* expression and potentiation effect was apparent in these studies (Verma et al., 2019).

Cytarabine (araC) is an antimetabolite anticancer agent clinically approved for use in many hematologic cancers including several subtypes of AML and in T-ALL. To the best of our knowledge, AKR1C3 does not metabolize drugs with an amide group. Pretreatment for 24 hours with either KV-49a or KV-49g followed by araC led to a >100-fold potentiation of the effect of araC in the KG1a cell line which shows a high expression of *AKR1C3*. Similar pretreatments in the HL-60 cell line resulted in 12-fold (with KV-49g) and 33-fold (with KV-49a) potentiation, whereas in the THP-1 cell line, 18-fold (with KV-49g) and 13-fold (with KV-49a) potentiation was observed. These results again show a correlation between *AKR1C3* expression and potentiation effect among the various cell lines (Verma et al., 2019).

In the high-risk pediatric T-ALL, a superior drug resistance profile is observed versus the related B-cell acute lymphoblastic leukemia. The expression and enzymatic activity of AKR1C3 was examined in a large cohort of patients with T-ALL which found a significant upregulation in resistant or poorly responding tumors to vincristine (Bortolozzi et al., 2018). In primary patient-derived T-ALL cell lines obtained at patient diagnosis (low *AKR1C3* expression) and at treatment relapse (high *AKR1C3* expression), cotreatment of KV-49a with daunorubicin resulted in 13-fold and 20-fold increase in potentiation, respectively. Pretreatment of KV-49a followed by daunorubicin greatly increased the potentiation effect. A >100-fold potentiation of daunorubicin was observed in both cell lines and reduced the daunorubicin IC_{50} from 0.005 μM and 0.07 μM at diagnosis and in relapsed cells, respectively, to < 0.001 μM in both cell lines. Similarly, in primary T-ALL cells taken at patient diagnosis, cotreatment of AKR1C3 inhibitor KV-49a with araC resulted in a 16-fold potentiation effect. In primary T-ALL cells taken at relapse, this effect was increased to >100 potentiation. Upon pretreatment of these cell lines with KV-49a followed by araC exposure, a >100-fold potentiation effect was achieved in both cell lines (Verma et al., 2019). Again, greater *AKR1C3* expression was correlated with a greater potentiation effect upon the use of an AKR1C3 inhibitor.

Etoposide is a DNA intercalator and topoisomerase II inhibitor that is clinically approved for the treatment of several forms of leukemia including AML. Pretreatment of an AKR1C3 inhibitor followed by exposure of cells to etoposide resulted in a 5.5-fold and 6.3-fold potentiation effect in HL-60 and KG1a AML cells, respectively, with a greater effect seen in higher AKR1C3-expressing cell lines. The higher potentiation effect seen in KG1a cells may be because of the higher expression of AKR1C3 in these cells (Verma et al., 2016).

Khanim et al. (2009) identified tetracycline as a potent and selective AKR1C3 inhibitor. Further studies showed that 4-methyl(de-dimethylamine)-tetracycline, a breakdown product of tetracycline, was a potent (IC_{50} = 0.51 μM) and somewhat selective AKR1C3 inhibitor. Despite the presence of 11-keto-prostaglandin reductase activity catalyzed by AKR1C3 in the KG1a AML cell line, 4-methyl(de-dimethylamine)-tetracycline did not exhibit antiproliferative activity up to 50 μM concentration in the AML cell lines HL60, KG1a, and K562. When combined with bezafibrate, no potentiation effect was noted (Khanim et al., 2009). However, medroxyprogesterone acetate, a pan-AKR1C inhibitor, provided enhanced reduction in cell viability when combined with bezafibrate in K562 and KG1a AML cell lines (Khanim et al., 2009). The ability of 6-medroxyprogesterone to have this affect implicates the action of AKR1C isoforms other than AKR1C3 in drug resistance.

C. Colorectal Cancer

As described earlier, purvalanol A and roscovitine exhibit good potency and selectivity for AKR1C3 inhibition, yielding K_i values of 5.5 μM and 1.4 μM, respectively in the AKR1C3 transfected human colon carcinoma (HCT116) cell line. A combination study with 0.1–1 μM daunorubicin and 5 μM purvalanol A or 10 μM roscovitine in AKR1C3 overexpressing HCT116 cells revealed excellent synergy compared with nontransfected HCT116 cells with a potentiation effect of the two drugs of 12.97 ± 6.26 and 17.06 ± 8.36-fold, respectively. These studies indicate that a

combination of purine analogs and daunorubicin resulted in excellent potentiation of activity and chemosensitized AKR1C3 expressing cell lines to the action of daunorubicin (Novotná et al., 2018a).

Buparlisib, a pyrimidine analog, is a known phosphoinositide 3-kinase (PI3K) inhibitor and is in clinical trial for the treatment of various cancers including leukemia. Buparlisib inhibits AKR1C3 with an IC_{50} of 9.5 μM and in AKR1C3 overexpressing HCT116 cells was evaluated for its inhibitory activity of AKR1C3-mediated intracellular reduction of daunorubicin, wherein a K_i value of 14 μM was determined using an enzymatic assay. This combination of buparlisib and daunorubicin results in decreased daunorubicin metabolism in AKR1C3 overexpressing human colon carcinoma cells (Bukum et al., 2019).

When AKR1C inhibitors are used to potentiate the effects of chemotherapeutic agents, some common features emerge. When AKR1C inhibitors and chemotherapeutic agents are given concurrently, potentiation is weak. However, more dramatic effects are seen if cancer cells are "primed by pretreatment" with the AKR1C inhibitors. This ordered addition of drug may have to be considered in the clinic for presensitization of patients. In some instances, the AKR1C inhibitors clearly inhibit the enzyme activity of the protein, but in other instances, this may not be the case, pointing toward a role in drug resistance that may be enzyme independent.

IX. Resistance Versus Disease Progression: Cause or Consequence

AKRs did not evolve to metabolize chemotherapeutic drugs, instead they have evolved to prevent cellular response to oxidative and electrophilic stress and may play a role in disease pathogenesis. For example, AKR1B10 converts all-*trans*-retinaldehyde to retinal and prevents the formation of retinoic acid and blocks retinoic acid signaling and cellular differentiation (Gallego et al., 2006, 2007). This pathogenic mechanism may account for its overexpression in many tumor types, e.g., nonsmall cell lung carcinoma and colon cancer (Hsu et al., 2001; Kropotova et al., 2014). Similarly, AKR1C3 also acts as a prostaglandin (PG) $F_{2\alpha}$ synthase and converts PGH_2 and 11-keto-PGD_2 to prostaglandins of the F series (Matsuura et al., 1998), which in turn can activate the FP1 receptor leading to activation of MAP-kinase and a pro-proliferative signal (Desmond et al., 2003; Penning, 2019). This pathogenic mechanism may account for its overexpression in many types of leukemia (Birtwistle et al., 2009; Verma et al., 2016; Bortolozzi et al., 2018). Finally, AKR1C1, AKR1C2 and AKR1C3 regulate the amount of ligands that can bind to steroid hormone receptors, and this prereceptor regulation of hormone action may contribute to the growth of hormone dependent malignancies (Penning et al., 2019). A consequence of this pathology, because of their ability to reduce carbonyl groups, can be chemoresistance.

X. Clinical Use of Nuclear Factor-Erythroid 2 p45-Related Factor 2 and AKR Inhibitors to Surmount Chemotherapeutic Drug Resistance

The ability of existing drugs to inhibit AKR1C isoforms selectively has been tested in some clinical trials. In the Nurses health cohort study, the use of both aspirin and nonaspirin based NSAIDs prolonged the survival of ovarian cancer patients where the primary chemotherapeutic agent was *cis*-platin and demonstrated the clinical utility of inhibiting an AKR1C isoform (Trabert et al., 2014). The clinically approved COX inhibitor INDO is currently in phase II clinical trials to surmount drug resistance to abiraterone acetate and enzalutamide mediated by AKR1C3 in castrate resistance prostate cancer (see NCT02849990 and NCT02935205). AKR1C3 has also been targeted in AML using a combination of 6-medroxyprogesterone acetate (a pan-AKR1C inhibitor) and bezafibrate (a PPARγ agonist) with the goal of preventing PGD_2 conversion to 11β-$PGF_{2\alpha}$ and preventing cellular proliferation (Murray et al., 2010); this approach has led to a phase II trial (Murray et al., 2019a). To move AKR inhibitors into oncologic practice to improve drug response to anticancer drugs, trials are in their infancy. Adaptive clinical trials that exploit overexpression of AKR isoforms could be considered so that personalized treatment based on tumor phenotype and AKR isoform specific inhibitors could be a treatment strategy. The use of NRF2 inhibitors would be another approach, but we need to better understand the mode of action of these agents when they do not bind to NRF2 directly.

XI. Conclusions

Resistance to anticancer drugs, whether they be cancer chemotherapeutic agents or anti-hormone therapeutics, remains a major factor limiting their use. For the former, there can also be dose-limiting toxicity. Identification of targets that mediate resistance and the development of inhibitors for these targets can prolong the benefit of existing agents, limit toxicity, and increase overall survival. This review establishes the case that members of the *AKR* gene superfamily are intimately involved in drug resistance and inhibitors may have a significant benefit in surmounting resistance and increasing drug sensitivity. In some instances, a battery of *AKR* genes can be involved, and thus, a balance exists between the use of isoform specific inhibitors and pan-inhibitors. Some of these inhibitors are existing drugs (e.g., indomethacin, 6-medroxyprogesterone acetate, flufenamic acid, purvalanol A, roscovitine, and buparlisib) and could be used for improved outcomes and can be tested in adaptive clinical trials.

In other cases where isoform specific inhibitors have been identified from enzyme screening and efficacy in cancer cell models has been established, more preclinical development of these agents is required. Knowing that the *AKR* genes are regulated by the NRF2-KEAP1 pathway identifies NRF2 inhibitors as another strategy to surmount drug resistance.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Penning, onnalagadda, Trippier, Rižner.

Acknowledgments

We acknowledge Dr. Jessica Murray and Nicole Kerstetter for their assistance in the preparation of figures. We would also like to dedicate this article to Dr. Amy Margaret Quinn, who completed her thesis on AKR1B10 in 2008 in Professor Penning's laboratory and died as a result of AML.

References

Adeniji AO, Chen M, and Penning TM (2013) AKR1C3 as a target in castrate resistant prostate cancer. *J Steroid Biochem Mol Biol* **137**:136–149.

Adeniji AO, Twenter BM, Byrns MC, Jin Y, Chen M, Winkler JD, and Penning TM (2012) Development of potent and selective inhibitors of aldo-keto reductase 1C3 (type 5 17β-hydroxysteroid dehydrogenase) based on N-phenyl-aminobenzoates and their structure-activity relationships. *J Med Chem* **55**:2311–2323.

Agyeman AS, Chaerkady R, Shaw PG, Davidson NE, Visvanathan K, Pandey A, and Kensler TW (2012) Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. *Breast Cancer Res Treat* **132**:175–187.

Bacolod MD, Lin SM, Johnson SP, Bullock NS, Colvin M, Bigner DD, and Friedman HS (2008) The gene expression profiles of medulloblastoma cell lines resistant to preactivated cyclophosphamide. *Curr Cancer Drug Targets* **8**:172–179.

Birtwistle J, Hayden RE, Khanim FL, Green RM, Pearce C, Davies NJ, Wake N, Schrewe H, Ride JP, Chipman JK, et al. (2009) The aldo-keto reductase AKR1C3 contributes to 7,12-dimethylbenz(a)anthracene-3,4-dihydrodiol mediated oxidative DNA damage in myeloid cells: implications for leukemogenesis. Mutat Res **662**:67–74.

Bortolozzi R, Bresolin S, Rampazzo E, Paganin M, Maule F, Mariotto E, Boso D, Minuzzo S, Agnusdei V, Viola G, et al. (2018) AKR1C enzymes sustain therapy resistance in paediatric T-ALL. *Br J Cancer* **118**:985–994.

Bukum N, Novotna E, Morell A, Hofman J, and Wsol V (2019) Buparlisib is a novel inhibitor of daunorubicin reduction mediated by aldo-keto reductase 1C3. *Chem Biol Interact* **302**:101–107.

Burczynski ME, Harvey RG, and Penning TM (1998) Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: oxidation of trans-7, 8-dihydroxy-7,8-dihydrobenzo[a]pyrene to the activated o-quinone metabolite benzo[a]pyrene-7,8-dione. *Biochemistry* **37**:6781–6790.

Burczynski ME, Lin HK, and Penning TM (1999) Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. *Cancer Res* **59**:607–614.

Burczynski ME, Sridhar GR, Palackal NT, and Penning TM (2001) The reactive oxygen species–and Michael acceptor-inducible human aldo-keto reductase AKR1C1 reduces the alpha,beta-unsaturated aldehyde 4-hydroxy-2-nonenal to 1,4-dihydroxy-2-nonene. *J Biol Chem* **276**:2890–2897.

Byrns MC, Duan L, Lee SH, Blair IA, and Penning TM (2010) Aldo-keto reductase 1C3 expression in MCF-7 cells reveals roles in steroid hormone and prostaglandin metabolism that may explain its over-expression in breast cancer. *J Steroid Biochem Mol Biol* **118**:177–187.

Byrns MC, Steckelbroeck S, and Penning TM (2008) An indomethacin analogue, N-(4-chlorobenzoyl)-melatonin, is a selective inhibitor of aldo-keto reductase 1C3 (type 2 3alpha-HSD, type 5 17beta-HSD, and prostaglandin F synthase), a potential target for the treatment of hormone dependent and hormone independent malignancies. *Biochem Pharmacol* **75**:484–493.

Canning P, Sorrell FJ, and Bullock AN (2015) Structural basis of Keap1 interactions with Nrf2. *Free Radic Biol Med* **88** (Pt B):101–107.

Chang WM, Chang YC, Yang YC, Lin SK, Chang PM, and Hsiao M (2019) AKR1C1 controls cisplatin-resistance in head and neck squamous cell carcinoma through cross-talk with the STAT1/3 signaling pathway. *J Exp Clin Cancer Res* **38**:245, DOI: 10.1186/s13046-019-1256-2.

Chen J, Emara N, Solomides C, Parekh H, and Simpkins H (2010) Resistance to platinum-based chemotherapy in lung cancer cell lines. Cancer Chemother Pharmacol **66**:1103–1111.

Chen CC, Chu CB, Liu KJ, Huang CY, Chang JY, Pan WY, Chen HH, Cheng YH, Lee KD, Chen MF, et al. (2013) Gene expression profiling for analysis acquired oxaliplatin resistant factors in human gastric carcinoma TSGH-S3 cells: the role of IL-6 signaling and Nrf2/AKR1C axis identification. *Biochem Pharmacol* **86**:872–887.

Chen J, Adikari M, Pallai R, Parekh HK, and Simpkins H (2008) Dihydrodiol dehydrogenases regulate the generation of reactive oxygen species and the development of cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother Pharmacol* **61**:979–987.

Chou TC and Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* **22**:27–55.

Chou TC (2010) Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res* **70**:440–446.

Ciaccio PJ and Tew KD (1994) cDNA and deduced amino acid sequences of a human colon dihydrodiol dehydrogenase. *Biochim Biophys Acta* **1186**:129–132.

Ciaccio PJ, Jaiswal AK, and Tew KD (1994) Regulation of human dihydrodiol dehydrogenase by Michael acceptor xenobiotics. *J Biol Chem* **269**:15558–15562.

Ciaccio PJ, Stuart JE, and Tew KD (1993) Overproduction of a 37.5-kDa cytosolic protein structurally related to prostaglandin F synthase in ethacrynic acid-resistant human colon cells. *Mol Pharmacol* **43**:845–853.

Cubillos-Angulo JM, Fukutan E, Cruz LAB, Arriaga MB, Lima JV, Andrade BB, Queiroz ATL, and Fukutani KF (2020) Systems biology analysis of publicly available transcriptomic data reveals a critical link between AKR1B10 gene expression, smoking and occurrence of lung cancer. *PLoS One* **15**:e0222552, DOI: 10.1371/journal.pone.0222552.

Deng HB, Adikari M, Parekh HK, and Simpkins H (2004) Ubiquitous induction of resistance to platinum drugs in human ovarian, cervical, germ-cell and lung carcinoma tumor cells overexpressing isoforms 1 and 2 of dihydrodiol dehydrogenase. *Cancer Chemother Pharmacol* **54**:301–307.

Deng HB, Parekh HK, Chow KC, and Simpkins H (2002) Increased expression of dihydrodiol dehydrogenase induces resistance to cisplatin in human ovarian carcinoma cells. *J Biol Chem* **277**:15035–15043.

Desmond JC, Mountford JC, Drayson MT, Walker EA, Hewison M, Ride JP, Luong QT, Hayden RE, Vanin EF, and Bunce CM (2003) The aldo-keto reductase AKR1C3 is a novel suppressor of cell differentiation that provides a plausible target for the non-cyclooxygenase-dependent antineoplastic actions of nonsteroidal anti-inflammatory drugs. *Cancer Res* **63**:505–512.

Dinkova-Kostova AT, Holtzclaw WD, and Kensler TW (2005) The role of Keap1 in cellular protective responses. *Chem Res Toxicol* **18**:1779–1791.

Dinkova-Kostova AT, Kostov RV, and Canning P (2017) Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. *Arch Biochem Biophys* **617**:84–93.

El-Kabbani O, Dhagat U, and Hara A (2011) Inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1). *J Steroid Biochem Mol Biol* **125**:105–111.

El-Kabbani O, Scammells PJ, Day T, Dhagat U, Endo S, Matsunaga T, Soda M, and Hara A (2010) Structure-based optimization and biological evaluation of human 20α-hydroxysteroid dehydrogenase (AKR1C1) salicylic acid-based inhibitors. *Eur J Med Chem* **45**:5309–5317.

Fabrizio FP, Sparaneo A, Centra F, Trombetta D, Storlazzi CT, Graziano P, Maiello E, Fazio VM, and Muscarella LA (2019) Methylation Density Pattern of *KEAP1* Gene in Lung Cancer Cell Lines Detected by Quantitative Methylation Specific PCR and Pyrosequencing. *Int J Mol Sci* **20**:2697.

Felsted RL, Gee M, and Bachur NR (1974) Rat liver daunorubicin reductase. An aldo-keto reductase. *J Biol Chem* **249**:3672–3679.

Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, Staffurth JN, North S, Vogelzang NJ, Saad F, et al.; COU-AA-301 Investigators (2012) Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol* **13**:983–992.

Flynn J, Jones J, Johnson AJ, Andritsos L, Maddocks K, Jaglowski S, Hessler J, Grever MR, Im E, Zhou H, et al. (2015) Dinaciclib is a novel cyclin-dependent kinase inhibitor with significant clinical activity in relapsed and refractory chronic lymphocytic leukemia. *Leukemia* **29**:1524–1529.

Gallego O, Belyaeva OV, Porté S, Ruiz FX, Stetsenko AV, Shabrova EV, Kostereva NV, Farrés J, Parés X, and Kedishvili NY (2006) Compartive functional analysis of human medium-chain dehydrogenases, short chain dehydrogenases/reductases and aldo-keto reductases with retinoids. *Biochem J* **399**:101–9.

Gallego O, Ruiz FX, Ardèvol A, Domínguez M, Alvarez R, de Lera AR, Rovira C, Farrés J, Fita I, and Parés X (2007) Structural basis for the high all-trans-retinaldehyde reductase activity of the tumor marker AKR1B10. *Proc Natl Acad Sci USA* **104**:20764–20769.

Gao A-M, Ke Z-P, Shi F, Sun GC, and Chen H (2013) Chrysin enhances sensitivity of BEL-7402/ADM cells to doxorubicin by suppressing PI3K/Akt/Nrf2 and ERK/Nrf2 pathway. *Chem Biol Interact* **206**:100–108.

Gewirtz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol* **57**:727–741.

Girardi E, César-Razquin A, Lindinger S, Papakostas K, Konecka J, Hemmerich J, Kickinger S, Kartnig F, Gürtl B, Klavins K, et al. (2020) A widespread role for SLC transmembrane transporters in resistance to cytotoxic drugs. *Nat Chem Biol* **16**:469–478.

Grossman RL, Heath AP, Ferretti V, Varmus HE, Lowy DR, Kibbe WA, and Staudt LM (2016) Toward a shared vision for cancer genomic data. N Engl J Med **375**:1109–1112.

Guo Y, Yu S, Zhang C, and Kong AN (2015) Epigenetic regulation of Keap1-Nrf2 signaling. *Free Radic Biol Med* **88** (Pt B):337–349.

Gupta SK, Singh P, Ali V, and Verma M (2020) Role of membrane-embedded drug efflux ABC transporters in the cancer chemotherapy. *Oncol Rev* **14**:448, 10.4081/oncol.2020.448

Hamid AR, Pfeiffer MJ, Verhaegh GW, Schaafsma E, Brandt A, Sweep FC, Sedelaar JP, and Schalken JA (2013) Aldo-keto reductase family 1 member C3 (AKR1C3) is a biomarker and therapeutic target for castration-resistant prostate cancer. *Mol Med* **18**:1449–1455.

Han J, Jun Y, Kim SH, Hoang HH, Jung Y, Kim S, Kim J, Austin RH, Lee S, and Park S (2016) Rapid emergence and mechanisms of resistance by U87 glioblastoma cells to doxorubicin in an in vitro tumor microfluidic ecology. *Proc Natl Acad Sci USA* **113**:14283–14288.

Hanada N, Takahata T, Zhou Q, Ye X, Sun R, Itoh J, Ishiguro A, Kijima H, Mimura J, Itoh K, et al. (2012) Methylation of the KEAP1 gene promoter region in human colorectal cancer. *BMC Cancer* **12**:66.

Harder B, Tian W, La Clair JJ, Tan A-C, Ooi A, Chapman E, and Zhang DD (2017) Brusatol overcomes chemoresistance through inhibition of protein translation. *Mol Carcinog* **56**:1493–1500.

Heibein AD, Guo B, Sprowl JA, Maclean DA, and Parissenti AM (2012) Role of aldo-keto reductases and other doxorubicin pharmacokinetic genes in doxorubicin resistance, DNA binding, and subcellular localization. *BMC Cancer* **12**:381, DOI: 10.1186/1471-2407-12-381.

Hintzpeter J, Seliger JM, Hofman J, Martin HJ, Wsol V, and Maser E (2016) Inhibition of human anthracycline reductases by emodin - A possible remedy for anthracycline resistance. *Toxicol Appl Pharmacol* **293**:21–29.

Hofland J, van Weerden WM, Dits NFJ, Steenbergen J, van Leenders GJ, Jenster G, Schröder FH, and de Jong FH (2010) Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. *Cancer Res* **70**:1256–1264.

Hofman J, Malcekova B, Skarka A, Novotna E, and Wsol V (2014) Anthracycline resistance mediated by reductive metabolism in cancer cells: the role of aldo-keto reductase 1C3. *Toxicol Appl Pharmacol* **278**:238–248.

Hsu NY, Ho HC, Chow KC, Lin TY, Shih CS, Wang LS, and Tsai CM (2001) Overexpression of dihydrodiol dehydrogenase as a prognostic marker of non-small cell lung cancer. *Cancer Res* **61**:2727–2731.

Hung JJ, Chow KC, Wang HW, and Wang LS (2006) Expression of dihydrodiol dehydrogenase and resistance to chemotherapy and radiotherapy in adenocarcinoma cells of lung. *Anticancer Res* **26** (4B):2949–2955.

Januchowski R, Zawierucha P, Andrzejewska M, Ruciński M, and Zabel M (2013) Microarray-based detection and expression analysis of ABC and SLC transporters in drug-resistant ovarian cancer cell lines. *Biomed Pharmacother* **67**:240–245.

Januchowski R, Zawierucha P, Ruciński M, Andrzejewska M, Wojtowicz K, Nowicki M, and Zabel M (2014) Drug transporter expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. *Biomed Pharmacother* **68**: 447-53.

Jez JM and Penning TM (2001) The aldo-keto reductase (AKR) superfamily: an update. *Chem Biol Interact* **130-132**:499–525.

Jordan MA (2002) Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr Med Chem Anticancer Agents* **2**:1–17.

Jung KA, Choi BH, Nam CW, Song M, Kim ST, Lee JY, and Kwak MK (2013) Identification of aldo-keto reductases as NRF2-target marker genes in human cells. *Toxicol Lett* **218**:39–49, DOI: 10.1016/j.toxlet.2012.12.026.

Kensler TW, Egner PA, Agyeman AS, Visvanathan K, Groopman JD, Chen JG, Chen TY, Fahey JW, and Talalay P (2013) Keap1-nrf2 signaling: a target for cancer prevention by sulforaphane. *Top Curr Chem* **329**:163–177.

Kerins MJ and Ooi A (2018) A catalogue of somatic NRF2 gain-of-function mutations in cancer. *Sci Rep* **8**:12846.

Khanim FL, Hayden RE, Birtwistle J, Lodi A, Tiziani S, Davies NJ, Ride JP, Viant MR, Gunther UL, Mountford JC, et al. (2009) Combined bezafibrate and medroxyprogesterone acetate: potential novel therapy for acute myeloid leukaemia. *PLoS One* **4**:e8147, DOI: 10.1371/journal.pone.0008147.

Knudsen KE and Penning TM (2010) Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer. *Trends Endocrinol Metab* **21**:315–324.

Ko BC, Ruepp B, Bohren KM, Gabbay KH, and Chung SS (1997) Identification and characterization of multiple osmotic response sequences in the human aldose reductase gene. J Biol Chem **272**:16431–16437.

Kropotova ES, Zinovieva OL, Zyryanova AF, Dybovaya VI, Prasolov VS, Beresten SF, Oparina NY, and Mashkova TD (2014) Altered expression of multiple genes involved in retinoic acid biosynthesis in human colorectal cancer. *Pathol Oncol Res* **20**:707–717.

Lanthaler K, Bilsland E, Dobson PD, Moss HJ, Pir P, Kell DB, and Oliver SG (2011) Genome-wide assessment of the carriers involved in the cellular uptake of drugs: a model system in yeast. *BMC Biol* **9**:70, DOI: 10.1186/1741-7007-9-70.

Lee EK, Regenold WT, and Shapiro P (2002) Inhibition of aldose reductase enhances HeLa cell sensitivity to chemotherapeutic drugs and involves activation of extracellular signal-regulated kinases. *Anticancer Drugs* **13**:859–868.

Li D, Ma S, and Ellis EM (2015) Nrf2-mediated adaptive response to methyl glyoxal in HepG2 cells involves the induction of AKR7A2. Chem Biol Interact **234**:366–371.

Li D, Ferrari M, and Ellis EM (2012a) Human aldo-keto reductase AKR7A2 protects against the cytotoxicity and mutagenicity of reactive aldehydes and lowers intracellular reactive oxygen species in hamster V79-4 cells. *Chem Biol Interact* **195**:25–34.

Li R, Evaul K, Sharma KK, Chang KH, Yoshimoto J, Liu J, Auchus RJ, and Sharifi N (2012b) Abiraterone inhibits 3β-hydroxysteroid dehydrogenase: a rationale for increasing drug exposure in castration-resistant prostate cancer. *Clin Cancer Res* **18**:3571–3579.

Liedtke AJ, Adeniji AO, Chen M, Byrns MC, Jin Y, Christianson DW, Marnett LJ, and Penning TM (2013) Development of potent and selective indomethacin analogues for the inhibition of AKR1C3 (Type 5 17β-hydroxysteroid dehydrogenase/prostaglandin F synthase) in castrate-resistant prostate cancer. *J Med Chem* **56**:2429–2446.

Liu C, Armstrong CM, Lou W, Lombard A, Evans CP, and Gao AC (2017) Inhibition of AKR1C3 activation overcomes resistance to abiraterone in advanced prostate cancer. *Mol Cancer Ther* **16**:35–44.

Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, Evans CP, and Gao AC (2015) Intracrine androgens and AKR1C3 activation confer resistance to Enzalutamide in prostate cancer. *Cancer Res* **75**:1413–1422.

Lyon RC, Li D, McGarvie G, and Ellis EM (2013) Aldo-keto reductases mediate constitutive and inducible protection against aldehyde toxicity in human neuroblastoma SH-SY5Y cells. *Neurochem Int* **62**:113–121.

Ma J, Luo DX, Huang C, Shen Y, Bu Y, Markwell S, Gao J, Liu J, Zu X, Cao Z, et al. (2012) AKR1B10 overexpression in breast cancer: association with tumor size, lymph node metastasis and patient survival and its potential as a novel serum marker. *Int J Cancer* **131**:E862–E871.

MacLeod AK, McMahon M, Plummer SM, Higgins LG, Penning TM, Igarashi K, and Hayes JD (2009) Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis* **30**:1571–1580.

Matsumoto R, Tsuda M, Yoshida K, Tanino M, Kimura T, Nishihara H, Abe T, Shinohara N, Nonomura K, and Tanaka S (2016) Aldo-keto reductase 1C1 induced by interleukin-1β mediates the invasive potential and drug resistance of metastatic bladder cancer cells. *Sci Rep* **6**:34625.

Matsunaga T, Hojo A, Yamane Y, Endo S, El-Kabbani O, and Hara A (2013) Pathophysiological roles of aldo-keto reductases (AKR1C1 and AKR1C3) in development of cisplatin resistance in human colon cancers. *Chem Biol Interact* **202**:234–242.

Matsunaga T, Okumura N, Saito H, Morikawa Y, Suenami K, Hisamatsu A, Endo S, and Ikari A (2020) **S**ignificance of aldo-keto reductase 1C3 and ATP-binding cassette transporter B1 in gain of irinotecan resistance in colon cancer cells. *Chem Biol Interact* **332**:109295, DOI: 10.1016/j.cbi.2020.109295.

Matsunaga T, Saito H, Endo S, Iguchi K, Soda M, El-Kabbani O, Hara A, and Ikari A (2016a) Roles of aldo-keto reductases 1B10 and 1C3 and ATP-binding cassette transporter in docetaxel tolerance. *Free Radic Res* **50**:1296–1308.

Matsunaga T, Suzuki A, Kezuka C, Okumura N, Iguchi K, Inoue I, Soda M, Endo S, El-Kabbani O, Hara A, et al. (2016b) Aldo-keto reductase 1B10 promotes development of cisplatin resistance in gastrointestinal cancer cells through down-regulating peroxisome proliferator-activated receptor-γ-dependent mechanism. *Chem Biol Interact* **256**:142–153.

Matsunaga T, Yamaguchi A, Morikawa Y, Kezuka C, Takazawa H, Endo S, El-Kabbani O, Tajima K, Ikari A, and Hara A (2014) Induction of aldo-keto reductases (AKR1C1 and AKR1C3) abolishes the efficacy of daunorubicin chemotherapy for leukemic U937 cells. *Anticancer Drugs* **25**:868–877.

Matsunaga T, Yamane Y, Iida K, Endo S, Banno Y, El-Kabbani O, and Hara A (2011) Involvement of the aldo-keto reductase, AKR1B10, in mitomycin-c resistance through reactive oxygen species-dependent mechanisms. *Anticancer Drugs* **22**:402–408.

Matsuura K, Shiraishi H, Hara A, Sato K, Deyashiki Y, Ninomiya M, and Sakai S (1998) Identification of a principal mRNA species for human 3alpha-hydroxysteroid dehydrogenase isoform (AKR1C3) that exhibits high prostaglandin D2 11-ketoreductase activity. *J Biochem* **124**:940–946.

McNamara KM and Sasano H (2019) The role of 17βHSDs in breast tissue and breast cancers. *Mol Cell Endocrinol* **489**:32–44.

Mindnich RD and Penning TM (2009) Aldo-keto reductase (AKR) superfamily: genomics and annotation. *Hum Genomics* **3**:362–370.

Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, Fleisher M, Sander C, Sawyers CL, and Scher HI (2012) Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Res* **72**:6142–6152.

Morell A, Čermáková L, Novotná E, Laštovičková L, Haddad M, Haddad A, Portillo R, and Wsól V (2020) Bruton's tyrosine kinase inhibitors ibrutinib and acalabrutinib counteract anthracycline resistance in cancer cells expressing AKR1C3. *Cancers (Basel)* **12**:3731, DOI: 10.3390/cancers12123731

Morikawa Y, Kezuka C, Endo S, Ikari A, Soda M, Yamamura K, Toyooka N, El-Kabbani O, Hara A, and Matsunaga T (2015) Acquisition of doxorubicin resistance facilitates migrating and invasive potentials of gastric cancer MKN45 cells through up-regulating aldo-keto reductase 1B10. *Chem Biol Interact* **230**:30–39.

Morsy A and Trippier PC (2020) Reversal of apalutamide and darolutamide aldo-keto reductase 1C3-mediated resistance by a small molecule inhibitor. *ACS Chem Biol* **15**:646–650.

Murray JA, Khanim FL, Hayden RE, Craddock CF, Holyoake TL, Jackson N, Lumley M, Bunce CM, and Drayson MT (2010) Combined bezafibrate and medroxyprogesterone acetate have efficacy without haematological toxicity in elderly and relapsed acute myeloid leukaemia (AML). *Br J Haematol* **149**:65–69.

Murray J, Pratt G, Jacob, A, Clark F, Blundred R, Fox S, Bishop R, Wheatley K, Khanim F, Bunce C, Drayson M. (2019a). Single arm phase II trials assessing the sfaety, compliance with and activity of bezafibrate and medroxyprogesterone acetate (BaP) therapy against myeloid and lymphoid cancers. *Contemp Clin Trials Commun* **14**:100361, DOI: 10.1016/j.conctc.2019.100361.

Murray JR, de la Vega L, Hayes JD, Duan L, and Penning TM (2019b) Induction of the antioxidant response by the transcription factor NRF2 increases bioactivation of the mutagenic air pollutant 3-nitrobenzanthrone in human lung cells. *Chem Res Toxicol* **32**:2538–2551.

Novotná E, Büküm N, Hofman J, Flaxová M, Kouklíková E, Louvarová D, and Wsól V (2018a) Roscovitine and purvalanol A effectively reverse anthracycline resistance mediated by the activity of aldo-keto reductase 1C3 (AKR1C3): A promising therapeutic target for cancer treatment. *Biochem Pharmacol* **156**:22–31.

Novotná E, Büküm N, Hofman J, Flaxová M, Kouklíková E, Louvarová D, and Wsól V (2018b) Aldo-keto reductase 1C3 (AKR1C3): a missing piece of the puzzle in the dinaciclib interaction profile. *Arch Toxicol* **92**:2845–2857.

Novotna R, Wsol V, Xiong G, and Maser E (2008) Inactivation of the anticancer drugs doxorubicin and oracin by aldo-keto reductase (AKR) 1C3. *Toxicol Lett* **181**:1–6.

Ohara H, Miyabe Y, Deyashiki Y, Matsuura K, and Hara A (1995) Reduction of drug ketones by dihydrodiol dehydrogenases, carbonyl reductase and aldehyde reductase of human liver. *Biochem Pharmacol* **50**:221–227.

Ohta T, Iijima K, Miyamoto M, Nakahara I, Tanaka H, Ohtsuji M, Suzuki T, Kobayashi A, Yokota J, Sakiyama T, et al. (2008) Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. *Cancer Res* **68**:1303–1309.

Park AL, Lin HK, Yang Q, Sing CW, Fan M, Mapstone TB, Gross NL, Gumerlock MK, Martin MD, Rabb CH, et al. (2010) Differential expression of type 2 3α/type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) in tumors of the central nervous system. *Int J Clin Exp Pathol* **3**:743–754.

Peng XC, Gong FM, Wei M, Chen X, Chen Y, Cheng K, Gao F, Xu F, Bi F, and Liu JY (2010) Proteomic analysis of cell lines to identify the irinotecan resistance proteins. *J Biosci* **35**:557–564.

Penning TM (2019) AKR1C3 (type 5 17β-hydroxysteroid dehydrogenase/ prostaglandin F synthase): Roles in malignancy and endocrine disorders. *Mol Cell Endocrinol* **489**:82–91.

Penning TM and Drury JE (2007) Human aldo-keto reductases: function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys* **464**:241–250.

Penning TM, Burczynski ME, Jez JM, Hung C-F, Lin H-K, Ma H, Moore M, Palackal N, and Ratnam K (2000) Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J* **351**:67–77.

Penning TM, Wangtrakuldee P, and Auchus RJ (2019) Structural and functional biology of aldo-keto reductase steroid-transforming enzymes. *Endocr Rev* **40**:447–475.

Pippione AC, Carnovale IM, Bonanni D, Sini M, Goyal P, Marini E, Pors K, Adinolfi S, Zonari D, Festuccia C, et al. (2018) Potent and selective aldo-keto reductase 1C3 (AKR1C3) inhibitors based on the benzoisoxazole moiety: application of a bioisosteric scaffold hopping approach to flufenamic acid. *Eur J Med Chem* **150**:930–945.

Pippione AC, Giraudo A, Bonanni D, Carnovale IM, Marini E, Cena C, Costale A, Zonari D, Pors K, Sadiq M, et al. (2017) Hydroxytriazole derivatives as potent and selective aldo-keto reductase 1C3 (AKR1C3) inhibitors discovered by bioisosteric scaffold hopping approach. *Eur J Med Chem* **139**:936–946.

Platt A, Xia Z, Liu Y, Chen G, and Lazarus P (2016) Impact of nonsynonymous single nucleotide polymorphisms on in-vitro metabolism of exemestane by hepatic cytosolic reductases. *Pharmacogenet Genomics* **26**:370–380.

Plebuch M, Soldan M, Hungerer C, Koch L, and Maser E (2007) Increased resistance of tumor cells to daunorubicin after transfection of cDNAs coding for anthracycline inactivating enzymes. *Cancer Lett* **255**:49–56.

Powell K, Semaan L, Conley-LaComb MK, Asangani I, Wu YM, Ginsburg KB, Williams J, Squire JA, Maddipati KR, Cher ML, et al. (2015) ERG/AKR1C3/AR constitutes a feed-forward loop for AR signaling in prostate Cancer Cells. *Clin Cancer Res* **21**:2569–2579.

Rajagopalan PT, Zhang Z, McCourt L, Dwyer M, Benkovic SJ, and Hammes GG (2002) Interaction of dihydrofolate reductase with methotrexate: ensemble and single-molecule kinetics. *Proc Natl Acad Sci USA* **99**:13481–13486.

Rizner TL, Smuc T, Rupreht R, Sinkovec J, and Penning TM (2006) AKR1C1 and AKR1C3 may determine progesterone and estrogen ratios in endometrial cancer. *Mol Cell Endocrinol* **248**:126–135.

Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, Rathkopf D, Shelkey J, Yu EY, Alumkal J, et al.; Prostate Cancer Foundation/Department of Defense Prostate Cancer Clinical Trials Consortium (2010) Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. *Lancet* **375**:1437–1446.

Selga E, Noé V, and Ciudad CJ (2008) Transcriptional regulation of aldo-keto reductase 1C1 in HT29 human colon cancer cells resistant to methotrexate: role in the cell cycle and apoptosis. *Biochem Pharmacol* **75**:414–426.

Shen Y, Zhong L, Johnson S, and Cao D (2011) Human aldo-keto reductases 1B1 and 1B10: a comparative study on their enzyme activity toward electrophilic carbonyl compounds. *Chem Biol Interact* **191**:192–198.

Shiiba M, Yamagami H, Yamamoto A, Minakawa Y, Okamoto A, Kasamatsu A, Sakamoto Y, Uzawa K, Takiguchi Y, and Tanzawa H (2017) Mefenamic acid enhances anticancer drug sensitivity via inhibition of aldo-keto reductase 1C enzyme activity. *Oncol Rep* **37**:2025–2032.

Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, and Balk SP (2006) Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* **66**:2815–2825.

Sun X, Li J, Li Y, Wang S, and Li Q (2020) Apatinib, a novel tyrosine kinase inhibitor, promotes ROS-dependent apoptosis and autophagy via the Nrf2/HO-1 pathway in ovarian cancer cells. *Oxid Med Cell Longev* **2020**:3145182, DOI: 10.1155/2020/3145182.

Suraweera L, Vasantha Rupasinhe HP, Dellaire G, and Xu G. (2020) Regulation of Nrf2/ARE pathway by dietary flavonoids: a friend or foe for cancer management? *Antioxidants (Basel)* **11**;**9**:973, DOI: 10.3390/antiox9100973.

Tebay L E, Roberston H, Durant ST, Vitale SR, Penning TM, Dinkova-Kostova AT, and Hayes JD (2015) Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic Biol Med* **88** (Pt B):108-46.

Tian H, Li X, Jiang W, Lv C, Sun W, Huang C, and Chen R (2016) High expression of AKR1C1 is associated with proliferation and migration of small-cell lung cancer cells. *Lung Cancer (Auckl)* **7**:53–61.

Tian Y, Zhao L, Zhang H, Liu X, Zhao L, Zhao X, Li Y, and Li J (2014) AKR1C3 overexpression may serve as a promising biomarker for prostate cancer progression. *Diagn Pathol* **9**:42–48.

Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, Brinton LA, Webb PM, Nagle CM, Jordan SJ, et al.; Australian Ovarian Cancer Study Group, Australian Cancer Study (Ovarian Cancer); Ovarian Cancer Association Consortium (2014) Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. *J Natl Cancer Inst* **106**:djt431, DOI: 10.1093/jnci/djt431.

Veitch ZW, Guo B, Hembruff SL, Bewick AJ, Heibein AD, Eng J, Cull S, Maclean DA, and Parissenti AM (2009) Induction of 1C aldoketoreductases and other drug dose-dependent genes upon acquisition of anthracycline resistance. *Pharmacogenet Genomics* **19**:477–488.

Verma K, Gupta N, Zang T, Wangtrakluldee P, Srivastava SK, Penning TM, and Trippier PC (2018) AKR1C3 inhibitor KV-37 exhibits antineoplastic effects and potentiates enzalutamide in combination therapy in prostate adenocarcinoma cells. *Mol Cancer Ther* **17**:1833–1845.

Verma K, Zang T, Gupta N, Penning TM, and Trippier PC (2016) Selective AKR1C3 inhibitors potentiate chemotherapeutic activity in multiple acute myeloid leukemia (AML) cell lines. *ACS Med Chem Lett* **7**:774–779.

Verma K, Zang T, Penning TM, and Trippier PC (2019) Potent and highly selective aldo-keto reductase 1C3 (AKR1C3) inhibitors act as chemotherapeutic potentiators in acute myeloid leukemia and T-cell acute lymphoblastic leukemia. *J Med Chem* **62**:3590–3616.

Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, and Talalay P (2004) Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci USA* **101**:2040–2045.

Wang C, Yan R, Luo D, Watabe K, Liao DF, and Cao D (2009) Aldo-keto reductase family 1 member B10 promotes cell survival by regulating lipid synthesis and eliminating carbonyls. *J Biol Chem* **284**:26742–26748.

Wang HW, Lin CP, Chiu JH, Chow KC, Kuo KT, Lin CS, and Wang LS (2007) Reversal of inflammation-associated dihydrodiol dehydrogenases (AKR1C1 and AKR1C2) overexpression and drug resistance in nonsmall cell lung cancer cells by wogonin and chrysin. *Int J Cancer* **120**:2019–2027.

Wang R, An J, Ji F, Jiao H, Sun H, and Zhou D (2008) Hypermethylation of the Keap1 gene in human lung cancer cell lines and lung cancer tissues. *Biochem Biophys Res Commun* **373**:151–154.

Wellestein A (2018) General principles of pharmacotherapy of cancer, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Brunton L, Dandan-Hilal R, and Knollmann BC eds) pp 1161-1236, John Wiley, New York.

Wsol V, Szotakova B, Martin HJ, and Maser E (2007) Aldo-keto reductases (AKR) from the AKR1C subfamily catalyze the carbonyl reduction of the novel anticancer drug oracin in man. *Toxicology* **238**:111–118.

Wu J and Wang D (2017) CLIC1 induces drug resistance in human choriocarcinoma through positive regulation of MRP1. *Oncol Res* **25**:863–871.

Yao D, Ding S, Burchell B, Wolf CR, and Friedberg T (2000) Detoxication of vinca alkaloids by human P450 CYP3A4-mediated metabolism: implications for the development of drug resistance. *J Pharmacol Exp Ther* **294**:387–395.

Yates MS, Kwak MK, Egner PA, Groopman JD, Bodreddigari S, Sutter TR, Baumgartner KJ, Roebuck BD, Liby KT, Yore MM, et al. (2006) Potent protection against aflatoxin-induced tumorigenesis through induction of Nrf2-regulated pathways by the triterpenoid 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole. *Cancer Res* **66**:2488–2494.

Zhao J, Xiang Y, Xiao C, Guo P, Wang D, Liu Y, and Shen Y (2014) AKR1C3 overexpression mediates methotrexate resistance in choriocarcinoma cells. *Int J Med Sci* **11**:1089–1097.

Zhong L, Shen H, Huang C, Jing H, and Cao D (2011) AKR1B10 induces cell resistance to daunorubicin and idarubicin by reducing C13 ketonic group. *Toxicol Appl Pharmacol* **255**:40–47.

Zhong T, Xu F, Xu J, Liu L, and Chen Y (2015) Aldo-keto reductase 1C3 (AKR1C3) is associated with the doxorubicin resistance in human breast cancer via PTEN loss. *Biomed Pharmacother* **69**:317–325.

Zhou C, Shen G, Yang F, Duan J, Wu Z, Yang M, Liu Y, Du X, Zhang X, and Xiao S (2020) Loss of AKR1C1 is a good prognostic factor in advanced NPC cases and increases chemosensitivity to cisplatin in NPC cells. *J Cell Mol Med* **24**:6438–6447.

Zu X, Yan R, Pan J, Zhong L, Cao Y, Ma J, Cai C, Huang D, Liu J, Chung FL, et al. (2017) Aldo-keto reductase 1B10 protects human colon cells from DNA damage induced by electrophilic carbonyl compounds. *Mol Carcinog* **56**:118–129.