Selective and Broad Anti-Tumor Activity of AKR1C3-Activated Prodrug AST-3424/OBI-3424

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Abstract #1220

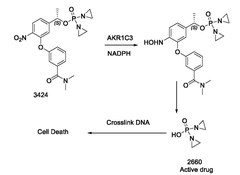
AST-3424/OBI-3424 (denoted by 3424) is a novel prodrug bis-alkylating agent activated by AKR1C3. AKR1C3 is overexpressed in many types of cancer, particularly in liver, non-small cell lung, gastric, renal and CRPC cancer. Currently 3424 is being studied in phase 1/2 clinical trials for the treatment of solid and hematologic cancers, and it represents potentially a novel, selective and broad anti-cancer agent. In this study, AKR1C3-dependent activation of 3424 was investigated *in vitro* using recombinant human AKR1C3. AKR1C3-dependent cytotoxicity of 3424 was determined in a wide range of human cancer cell lines with different AKR1C3 expression levels. In addition, anti-tumor activity of 3424 was also investigated in a broad panel of CDX and PDX models. AKR1C3-dependent activation of prodrug 3424 was evident by monitoring the decrease of 3424 and generation of the active form, 2660. Kinetic analysis indicated that AKR1C3 exhibited higher catalytic efficiency towards 3424 compared to the physiological substrates. There was a strong correlation between 3424 cytotoxic potency and AKR1C3 expression. The racemic mixture induced DNA cross-linking in a concentration dependent manner. Tumor growth inhibition of 3424 was shown to be better than or comparable to the standard of care chemotherapy at clinically achievable doses as a single agent in various CDX models with high expression of AKR1C3 including liver HepG2, lung H460, gastric SNU-16, kidney A498, castration-resistant prostate VCaP cancers. The excellent anti-tumor efficacy of 3424 was further demonstrated in PDX models that have high level of AKR1C3 expression, but not in a model with low level of AKR1C3 expression. In the combination therapy, we showed that 3424 could enhance the efficacy of the standard care of chemotherapy in the CDX models. The results described here highlight that 3424 exhibits AKR1C3-dependent cytotoxicity *in vitro* and anti-tumor activity *in vivo* in a wide range of human cancer types, which support further development of 3424 as an anti-cancer agent for treating different types of cancers and the use of AKR1C3 as a biomarker to profile cancer patients and further guide patient selection for therapy with 3424.

Background

Aldo-keto reductase 1C3 (AKR1C3) is one member of the 15 gene families of aldo-keto reductases (AKRs) [1]. AKR1C3 shares high sequence homology with the related human AKR1C family, including AKR1C1, AKR1C2, and AKR1C4. Many studies have demonstrated that AKR1C3 is overexpressed in many malignant solid and hematologic tumors, especially in hepatoma, bladder, renal, and gastric cancers [2]. AKR1C3 upregulation in cancer is reported to be associated with metastasis of cancer, poor prognosis and a low survival rate [3]. In addition, many types of treatment resistance are attributed to the overexpression of AKR1C3 [4]. One study has shown that high expression of AKR1C3 is associated with the failure of PD-1–targeted therapies in PD-L1 positive patients with advanced renal cell carcinoma (RCC) [5]. Due to tumor-specific overexpression of AKR1C3, the design of AKR1C3-activated prodrugs becomes an attractive approach to specifically target cancer. One such example is the AKR1C3-activated prodrug, PR104, which exhibited good anti-tumor activity *in vitro* and *in vivo* [6]. Prodrug 3424 is currently under development by Ascentawits Pharmaceuticals, LTD in Asian countries and by OBI Pharma, Inc. in countries outside Asia for the treatment of malignant tumors. Prodrug 3424 is currently being investigated in multiple Phase I clinical trials in the US (NCT04315324 & NCT03592264) and in China (CXHL1900137 & CXHL2000263) to treat human cancer. Due to the high expression of AKR1C3 in tumors, prodrug 3424 is designed to be specifically activated in tumors but spared in normal cells which express low levels of AKR1C3 to achieve tumor-specific targeting. Furthermore, tumor-selective activation of 3424 is distinguishable from non-selective traditional alkylating agents, such as cyclophosphamide and ifosfamide, indicating that 3424 has the potential to become a broad-spectrum, highly selective anti-tumor drug. Prodrug 3424 was reported to exhibit potent efficacy against preclinical models of T-ALL *in vitro* and *in vivo* [7, 8].

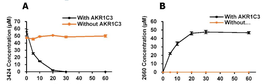
Results

Mechanism of 3424 activation



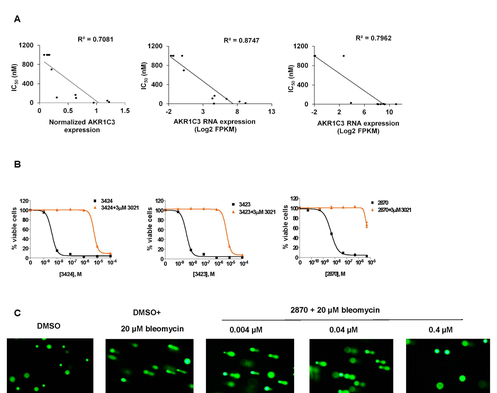
3424 activation. Schema of 3424 reductive activation pathway.

AKR1C3-dependent 3424 activation



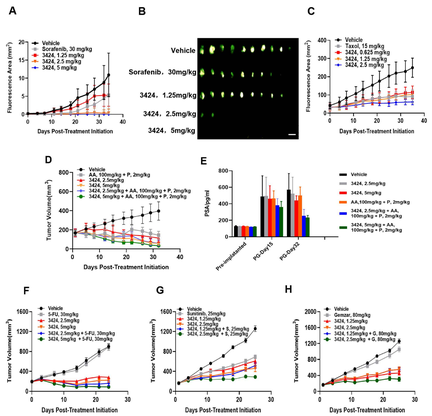
AKR1C3-dependent 3424 activation. The activation of 3424 by AKR1C3 was monitored by the reduction of 3424 and the generation of the active form 2660, using LC/MS-MS. (A) 3424 reduction; (B) 2660 production

AKR1C3-dependent 3424 activity



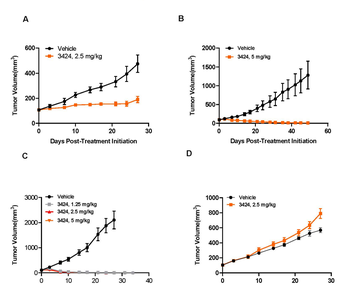
AKR1C3-dependent *in vitro* activity of 3424. A. Correlation between AKR1C3 protein expression and 3424 IC_{50} in liver cancer cells (left); Correlation between AKR1C3 RNA expression and 3424 IC_{50} in liver cancer cells (middle); Correlation between AKR1C3 RNA expression with 3424 IC_{50} in NSCLC cancer cells (right). B. AKR1C3-specific inhibitor 3021 efficiently inhibited cytotoxicity of 3424 (left), 3423 (middle) and racemic mixture 2870 (right). The data are the representative of three independent experiments. C. Compound 2870 induced concentration-dependent DNA cross-linking. The data are the representative images of cell nuclei stained with SYBR Green after electrophoresis of two independent experiments. Original magnification: 10 ×, scale bar: 100 μm.

Anti-tumor activity of 3424 in CDX models



***In vivo* anti-tumor efficacy of 3424 in CDX models.** Tumor growth of 3424 in Hep G2 (A & B), H460 (C), VCaP (D). Measurement of serum prostate specific antigen (PSA) in VCap (E). Tumor growth of 3424 in SNU-16 (F) and A498 (G & H). AA, abiraterone acetate; P, prednisolone; 5-FU, 5-flurouracil; S, sunitinib; G, gemcitabine

Anti-tumor Activity of 3424 in PDX models



***In vivo* anti-tumor activity of 3424 against a panel of PDXs.** A. Tumor growth of 3424 in pancreatic cancer PA1280. B. Tumor growth of 3424 in gastric cancer GA6201. C. Tumor growth of 3424 in lung cancer LU2505 with higher AKR1C3 expression. D. Tumor growth of 3424 in lung cancer LU2057 with low AKR1C3 expression. Animals were monitored daily and tumor growth was quantified twice a week. Data are expressed as Mean ± SEM of 5-6 animals per group.

Translational Significance

AST-3424/OBI-3424 (denoted by 3424) is a novel prodrug bis-alkylating agent activated by AKR1C3 and is currently being investigated in multiple clinical trials for the treatment of solid and hematologic cancers. Activation of 3424 is human AKR1C3-specific. There is a strong correlation between 3424 *in vitro* cytotoxicity and AKR1C3 expression at levels of protein and RNA in a wide range of human cancer cell lines. In addition, we show excellent *in vivo* anti-tumor activity of 3424 at clinically achievable doses in a broad panel of CDX and PDX models with high expression of AKR1C3. Of note, 3424 shows remarkable *in vivo* efficacy towards liver, gastric, kidney, lung, pancreatic, and castration-resistant prostate cancers. The AKR1C3-dependent activity of 3424 has served as the basis for ongoing and future clinical trials that target cancer cells specifically and as a biomarker to profile cancer patients and further guide patient selection for therapy with 3424.

Conclusions

- AKR1C3-dependent 3424 activation
- AKR1C3-dependent 3424 cytotoxicity
- Concentration-dependent DNA cross-linking induced by 3424
- Excellent anti-tumor activity in CDX models as a monotherapy or in combination with standard of care chemotherapy
- Excellent anti-tumor activity in PDX models
- The data support further development of 3424 as an anti-cancer agent for treating different types of cancers and the use of AKR1C3 as a biomarker to profile cancer patients and further guide patient selection for therapy with 3424.

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