

**Publication in Journal of Medicinal Chemistry Describing
the Discovery of BTK Inhibitor AS-1763**

Carna Biosciences, a clinical-stage biopharmaceutical company focusing on the discovery and development of innovative therapies to treat serious unmet medical needs, announces that the research paper on the discovery of AS-1763, a next generation BTK inhibitor discovered by Carna, has been published in Journal of Medicinal Chemistry.

AS-1763 is an investigational small molecule drug designed to non-covalently inhibit Bruton's tyrosine kinase (BTK) in a highly selective manner. According to the preclinical data, the compound is capable of potently inhibiting both wild and C481S mutant BTK, and AS-1763 is currently under development for treating patients with chronic lymphocytic leukemia (CLL) and other B cell malignancies with acquired resistance to covalent BTK inhibitors.

Carna is conducting clinical studies of two highly selective BTK inhibitors, AS-0871 and AS-1763, and the publication in the Drug Annotations Series section of Journal of Medicinal Chemistry describes Carna's strategy and scientific findings to identify AS-1763.

Abstract of the paper

BTK is a nonreceptor tyrosine kinase belongs to the Tec family and expressed in hematopoietic cells mainly in B-cells and myeloid cells. BTK plays a crucial role in B cell antigen receptor (BCR) signaling which is essential for B cell development. Therefore, BTK has been recognized as a validated therapeutic target for B cell malignancies including chronic lymphocytic leukemia (CLL). First generation covalent BTK inhibitors, represented by ibrutinib, have been appreciated as a promising targeted therapy for patients with B cell malignancies. However, the emergence of clinical resistance to these covalent BTK inhibitors is becoming serious concerns. The most common mutation, BTK C481S was found in patients with acquired resistance to ibrutinib. Therefore, it is considered that the primary resistance mechanism for the first generation BTK inhibitors is mainly due to the C481S mutation, which prevents the covalent binding of the inhibitors and diminishes the inhibitory activities. There is a high unmet medical need for new therapeutic approaches to overcome the BTK C481S-mediated resistance, and non-covalent and reversible BTK inhibitors would be the most promising option to treat patients with BTK C481S mutation (Figure 1).

We have previously reported Carna's proprietary drug discovery technology⁽¹⁾ to screen compounds, which strongly inhibit an unactivated form of BTK (BTK[U]) than an activated form of BTK (BTK[A]), and identified a series of novel selective BTK inhibitors including our clinical compound, AS-0871 using this technology.⁽²⁾ We found that these compounds showed strong efficacy in various allergy and autoimmune disease models while anti-proliferative potency of such compounds in BTK inhibitor-sensitive OCI-Ly10 cells (human Diffuse large B-cell lymphoma (DLBCL) cell line) was moderate.

In order to generate a novel BTK inhibitor targeting BTK C481S mutant with potent anti-proliferative efficacy in OCI-Ly10 cells, we implemented the optimization study of compound **6** (identified in the previous

paper⁽²⁾) by measuring inhibitory potency for BTK[A] as the primary criteria (Figure 2). After scaffold hopping from **6** and the subsequent structural optimization, compound **13f** was identified as a potent and selective inhibitor of BTK with improved anti-proliferative activity for OCI-Ly10 cells. The compound **13f** showed significant anti-tumor effects in two xenograft mouse models bearing OCI-Ly10 and BTK[C481S] knock-in OCI-Ly10 cells. (Figure 3). In addition, compound **13** showed favorable ADMEPK profiles in various animal species, predicting its good human PK profile. Based on these preclinical results, we advanced compound **13f** to phase 1 clinical trials as AS-1763.

Figure 1. Activity of AS-1763 against ibrutinib resistant B-cell cancers

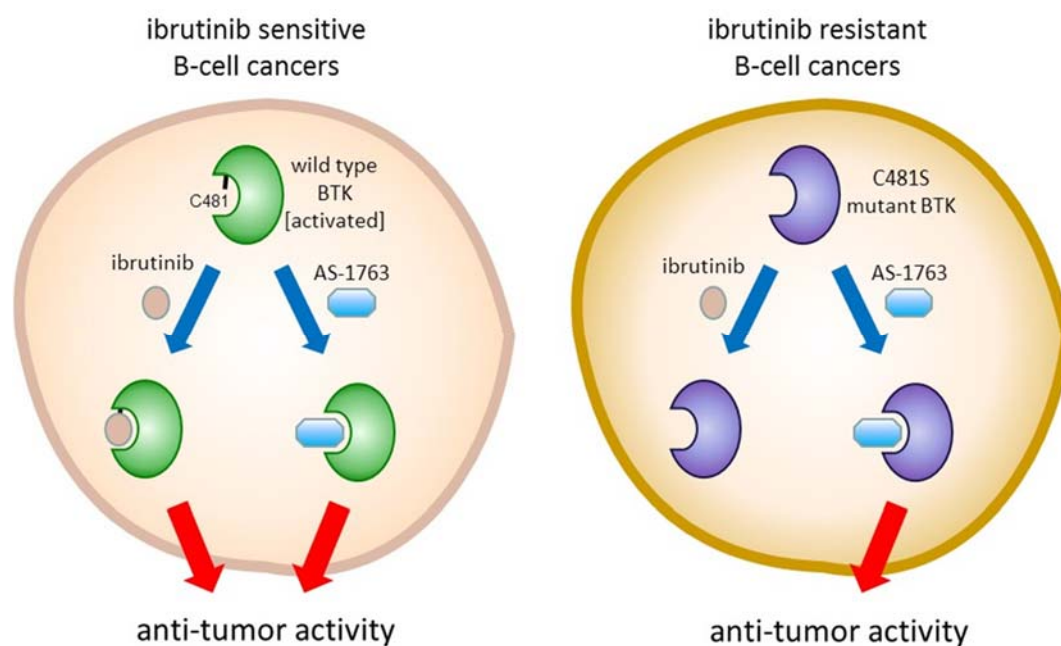


Figure 2. Strategy for Structural optimization of **6**

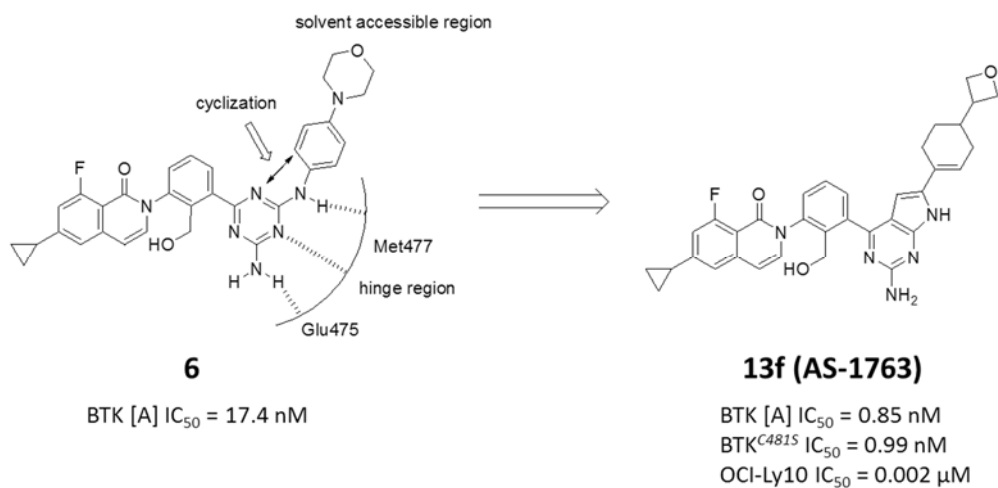
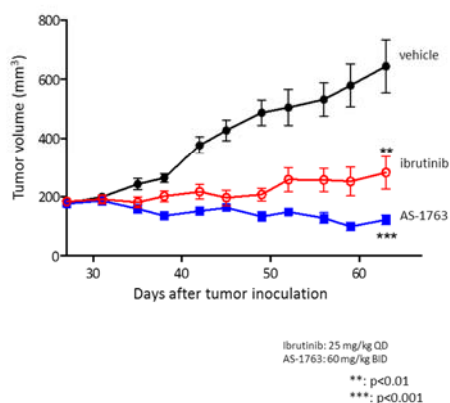
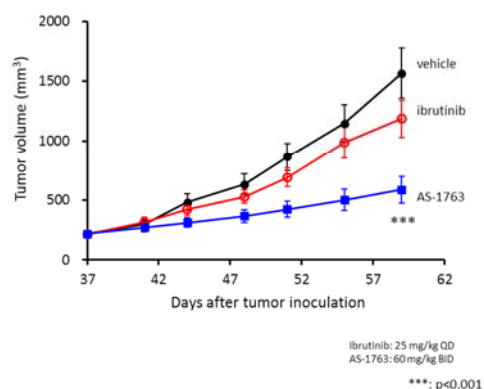


Figure 3. Anti-tumor effects of AS-1763 on OCI-Ly10 and BTKC481S knock-in OCI-Ly10 tumor xenograft models.

◆ In vivo antitumor effects of AS-1763 on human B-cell non-Hodgkin lymphoma cell line, OCI-LY10 tumor xenograft mouse model (n=8-10)



◆ In vivo antitumor effects of AS-1763 on ibrutinib-resistant BTK^{C481S} knock-in OCI-LY10 tumor xenograft mouse model (n=11)



References

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- (2) W. Kawahata, T. Asami, T. Kiyoi, T. Irie, H. Taniguchi, Y. Asamitsu, T. Inoue, T. Miyake, M. Sawa, *J. Med. Chem.* 2018, 61(19), 8917-8933.

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About AS-1763

AS-1763 is a highly selective, orally bioavailable, non-covalent inhibitor of both the wild type and C481S mutant Bruton's tyrosine kinases (BTK) for the treatment of chronic lymphocytic leukemia (CLL) and other B cell malignancies. First generation covalent BTK inhibitors including ibrutinib are key therapeutic options for patients with B cell malignancies. However, patients are reported to develop resistance during the treatment due to substitution of cysteine residue at 481 position with serine (C481S mutation) in BTK, which prevents the covalent binding of the first generation irreversible BTK inhibitors. In in vitro experiments, AS-1763 significantly abrogates cell proliferation in both wild type and C481S mutant BTK lymphoma cells, strongly suggesting AS-1763 will be a new therapeutic option for treating patients with B cell malignancies both having wild type and C481S mutation in BTK.

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