OATD-02 validates the benefits of pharmacological inhibition of arginase 1 and 2 in cancer

M. M. Grzybowski*, R. Błaszczyk, P. Pomper, P. S. Stańczak, B. Borek, A. Gzik, J. Nowicka, K. Jędrzejczak, T. Rejczak, N. C. Güner-Chalimoniuk, A. Kikulska, M. Mlącki, J. Pęczkowicz-Szyszka, J. Olczak, K. Dzwonek, P. Dobrzański, A. Gołębiowski & Z. Zasłona

Molecure SA (Żwirki i Wigury 101, 02-089 Warsaw, Poland)

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BACKGROUND

ARG1 and ARG2 are drivers of multiple immunosuppressive mechanisms and tumour-specific metabolic adaptations

Arginases play essential roles in metabolic pathways, determining the fitness of both immune and tumour cells. Along with the previously validated role of ARG1 in cancer, the particular significance of ARG2 as a therapeutic target has emerged as its levels correlate with malignant phenotype and poor prognosis. These observations unveil arginases, and specifically ARG2, as well-validated and promising therapeutic targets. OATD-02, a new boronic acid derivative, is the only dual inhibitor, which can address the benefits of pharmacological inhibition of arginase 1 and 2 in cancer.

OATD-02 inhibits both extracellular and intracellular arginases and restores the effective antitumor immune response

OATD-02 inhibits both extracellular and intracellular ARG1 and ARG2, interfering with multiple immunosuppressive mechanisms, and thus, restoring the effective antitumor immune response (Fig. 1). OATD-02 may block the extracellular ARG1 secreted by myeloid-derived suppressor cells (MDSCs) and tumour cells, increasing the availability of L-arginine for effector T and NK cells, but most significantly, the molecule is able to target the intracellular arginases, i.e., cytoplasmic ARG1 and mitochondrial ARG2, that is crucial for the function of immunosuppressive cells, like Tregs, but also negatively regulate the fitness of cytotoxic $CD8^+$ T cells. The unique properties of OATD-02 allow it to act also on ARG1 packed into the extracellular vesicles (EVs), as well as to counteract ARG2-dependent metabolic adaptations specific for both cancerous and cancer-associated cells, such as cancer-associated fibroblasts (CAFs).

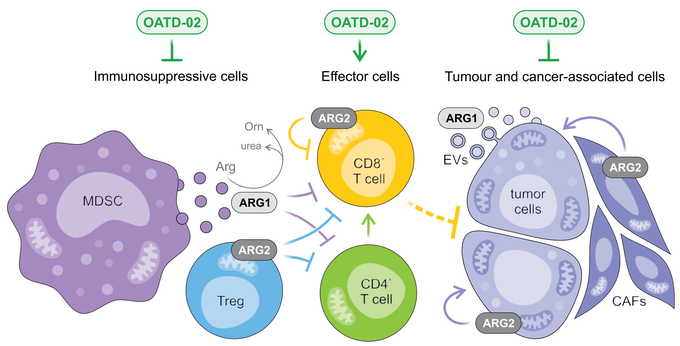

Figure 1. Arginase 1 and 2 are involved in multiple mechanisms supporting the growth of tumour cells. OATD-02, a small-molecule arginase inhibitor, may block both ARG1 and mitochondrial ARG2 exhibiting unique pharmacological properties. OATD-02 restores the effector functions of T and NK cells, modulate the suppressive role of Tregs and directly limit the growth of ARG2-dependent cancerous cells. MDSC - myeloid-derived suppressor cells, EVs - extracellular vesicles, CAFs - cancer-associated fibroblasts, Orn - ornithine.

METHODS

OATD-02 was synthetized at Molecure SA. The ref. ARGi (numidargistat) was purchased from ChemieTek (Indianapolis, USA). The activity of the tested compounds was determined *in vitro* using recombinant human ARG1 and ARG2, as well as in cell-based assays with primary human hepatocytes and murine BMDMs (bone marrow-derived M2-polarized macrophages). To assess the antiproliferative effect of OATD-02 on K562 cells, long-term cell culture was established; the medium was not replaced for two weeks and the cell density/viability was determined daily. *In vivo* antitumour activity was evaluated in murine syngeneic models of colorectal (CT26) and kidney (Renca) carcinomas, as well as in a xenograft leukemia model (K562). All animal procedures were approved by appropriate local ethics committees.

RESULTS

OATD-02 is a highly active dual ARG1/ARG2 inhibitor

OATD-02 proved to be a highly potent dual ARG1/ARG2 inhibitor (Fig. 2A-B). The difference in activities of the tested compounds was even more profound when we evaluated their intracellular activities towards arginase-expressing immune cells (Fig. 2C) – bone marrow-derived M2-polarized macrophages were used to surrogate the myeloid suppressor cells infiltrating the TME. Importantly, the inhibition of the liver intracellular arginase was low for both compounds when tested on primary hepatocytes (Fig. 2D).

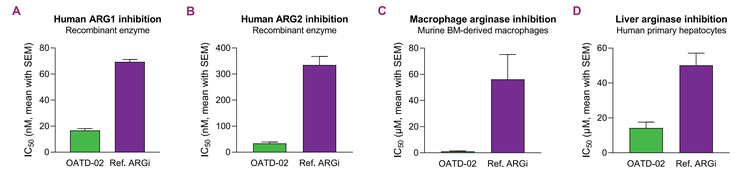

Figure 2. *In vitro* assays with recombinant human ARG1 (**A**) and ARG2 (**B**) enzymes mimic the extracellular activity of OATD-02, while cell-based assays with murine M2-polarized BMDMs (**C**) and platable human primary hepatocytes (**D**) reveal its potential in inhibiting intracellular arginases.

OATD-02 showed superior efficacy in combination therapy

In the syngeneic CT26 model, OATD-02 significantly inhibited the tumour growth (TGI 48% vs 28% for the ref. ARGi with a predominant extracellular activity, Fig. 3A-B). Both arginase inhibitors were dosed at 100 mg/kg (PO, BID from day 1 post-inoculation). Observed efficacy was reflected by the increase of the engogenous arginine levels (Fig. 3C). Encouraged by these results, we tested OATD-02 in a combination therapy with immune checkpoint inhibitor (anti-PD-L1 antibody) and an IDO1 inhibitor (epacadostat), as shown in Fig. 4A-B. The addition of OATD-02 to the anti-PD-L1/epacadostat combination led to a superior efficacy (TGI 81% vs 42% for the dual treatment) observed for this triple combination therapy, giving a rational for combinatorial application of arginase inhibitors in the clinical development.

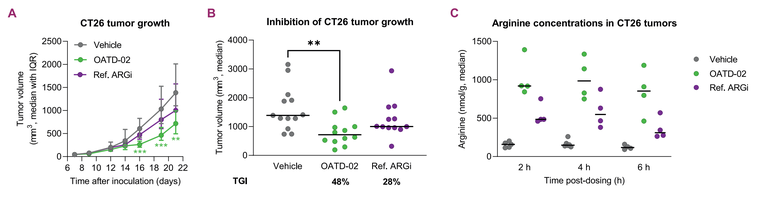

Figure 3. OATD-02 significantly reduced the growth of CT26 tumours. BALB/c mice were inoculated with CT26 cells and dosed with OATD-02 or the ref. ARGi (numidargistat) at 100 mg/kg (PO, BID from day 1); **A** - kinetics of the tumor growth, **B** - final tumour volume measurements with TGI index (tumour growth inhibition), **C** - measured PD effect (L-arginine) in the tumour homogenates; Kruskal-Wallis test with Dunn's multiple comparisons test was used.

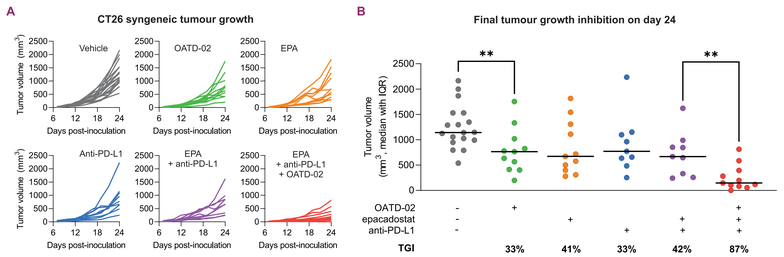

Figure 4. OATD-02 significantly improved the efficacy of a dual anti-PD-L1/epacadostat therapy. BALB/c mice were inoculated with CT26 cells and dosed with OATD-02 (50 mg/kg, PO, BID from day 1) or epacadostat (EPA, 30 mg/kg, PO, BID from day 1) or anti-PD-L1 antibody (2.5 mg/kg, IP, QD at days: 8, 10, 12, 14, 16); **A** - kinetics of the tumour growth, **B** - final tumour volume measurements with TGI index (tumour growth inhibition); U Mann-Whitney test was used for statistical analysis.

OATD-02 altered the TME of ARG2-positive Renca tumours

In the next step, we evaluated the activity of OATD-02 in the Renca tumour model, which is characterized by a strong infiltration of Tregs and MDSCs, as well as ARG2 expression by tumour cells. The monotherapy with OATD-02 dosed at 75 mg/kg (PO, BID from day 1) resulted in partial inhibition of the tumour growth (TGI 31%, Fig. 5A) correlated with a reversal of the immunosuppression in the TME (Fig. 5B), as evidenced by decreased levels of Tregs, MDSCs and neutrophils, as well as by an increase in the $CD8^+$/Treg ratio.

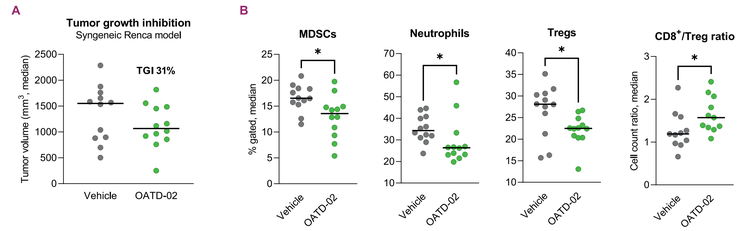

Figure 5. OATD-02 exhibited moderate therapeutic efficacy in the syngeneic Renca tumour model while changing the immunosuppressive TME. Renca tumour cells were subcutaneously implanted to BALB/c mice and OATD-02 was dosed orally at 75 mg/kg (BID from day 1); **A** - final tumour volume measurements with the tumour growth inhibition (TGI) index, **B** - significant changes in the immune infiltration of the tumours; U Mann-Whitney test was used for statistical analysis.

OATD-02 showed direct antitumour effect on human leukemic cells

To determine if OATD-02 can act independantly of the immune response by inhibiting the intracellular ARG2, we tested the effect of OATD-02 on the survival of leukemic K562 cells (Fig. 6B) with a strong ARG2 expression (Fig. 6A). OATD-02-treated cells were dying faster and in a dose-dependent manner. This encouraged us to test OATD-02 in a xenograft model using athymic nude mice. As shown in Fig. 6C-D, the growth of K562 tumours was significantly inhibited by OATD-02. These results were reproduced in mice with severe immunodeficiency including NK cells (not shown), imlying that OATD-02 directly inhibits the growth of ARG2-positive K562 tumours, most likely via interfering with specific metabolic adaptations.

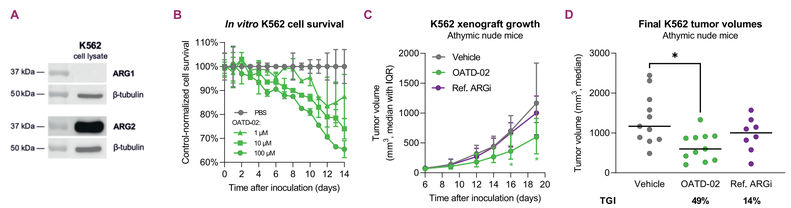

Figure 6. OATD-02 showed direct antitumour efficacy in the CML model using human K562 cells. Immunodeficient mice were inoculated with K562 cells and dosed orally with OATD-02 at 50 mg/kg or the ref. ARGi at 100 mg/kg (PO, BID from day 6); **A** - detection of ARG1/2 expression by SDS-PAGE/WB, **B** - OATD-02 dose-dependent effect on K562 cell survival, **C** - K562 xenograft growth kinetics, **D** - final measurements of tumour volume; statistical analysis was done with the Kruskal-Wallis and Dunn's multiple comparison tests.

- Our results reveal the benefits of inhibiting both ARG1/ARG2 over ARG1 alone
- OATD-02 showed immunomodulatory activity and therapeutic efficacy in multiple syngeneic tumour models with a superior effect in combination therapies
- OATD-02 may alter the tumour-specific ARG2-dependent metabolic adaptations and exert a direct antitumour efficacy, especially in hypoxic tumours
- OATD-02 enters phase I clinical trials in cancer patients this year

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PRE-CLINICAL AND CLINICAL DEVELOPMENT OF ARGINASE INHIBITOR FOR APPLICATION IN ANTI-CANCER IMMUNOTHERAPY

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