DEVELOPMENT OF OAT-1746: A NOVEL ARGINASE 1 AND 2 INHIBITOR FOR CANCER IMMUNOTHEPAPY

Stanczak PS[1], Grzybowski MM[1], Wolska P[1], Zdziarska AM[1], Mazurkiewicz M[1], Blaszczyk R[1] Nowicka J[1], Sosnowska A[2], Ramji K[2], Nowis D[3], Golab J[2], Golebiowski A[1], Dobrzanski P[1], Dzwonek K[1]

[1] OncoArendi Therapeutics SA, Warsaw, Poland; [2] Department of Immunology, Medical University of Warsaw, Warsaw, Poland, [3] Genomic Medicine, Medical University of Warsaw, Warsaw, Poland

BACKGROUND

Clinical success of PD-1/PD-L1 and CTLA-4 inhibitors demonstrated that reactivation of anti-tumor immunity provides strong clinical benefits including curative responses. However, only a fraction of patients achieved long-lasting therapeutic benefits prompting efforts to target additional effectors of anti-tumor immunity. Evidence from preclinical and clinical studies indicated that interference with multiple immune checkpoints provides extended therapeutic responses. Depletion of arginine inhibits proliferation and activation of T and NK cells and is an important mechanism of immunosuppression. High arginase activity has been found in patients with a wide spectrum of cancers, both in plasma and in tumors and correlated with a poor prognosis. We have developed potent and selective dual ARG1/2 inhibitors and demonstrated a strong anti-tumor efficacy of the lead compound OAT-1746 as a monotherapy and in combinations with other immunomodulators.

METHODS

The IC_{50} values of the compounds were determined against rARG1/2. M2-polarized, bone marrow-derived murine macrophages and CHO-K1 cells transfected with human ARG1 were used to assess the cellular activity. Murine and human $CD4^+/CD8^+$ T cells were negatively isolated and incubated with anti-CD3/CD28 beads to trigger proliferation, CD3ζ levels were measured. The *in vivo* antitumor efficacy was evaluated in syngeneic mouse models after oral administration.

RESULTS

► We have developed potent, selective, orally active inhibitors of arginase 1 and 2. Our lead compound OAT-1746 is a low nanomolar dual inhibitor of ARG1 and ARG2 with a potent cellular activity.

Table 1. *Activity of OAT-1746 against recombinant human (h), mouse (m) and rat (r) ARG1 and ARG2 and against ARG1 in cell-based assays*

Enzymatic assays	IC_{50}
Recombinant hARG1	32 nM
Recombinant hARG2	75 nM
Recombinant mARG1	50 nM
Recombinant rARG1	73 nM

Cell-based assays	IC_{50}
BM-derived M2-polarized mouse macrophages	55 nM
CHO-K1 cells trasfected with pCMV3-hARG1	34 nM

► OAT-1746 reversed ARG1-mediated suppression of human and murine $CD4^+$ and $CD8^+$ T cell proliferation and of CD3ε and CD3ζ expression.

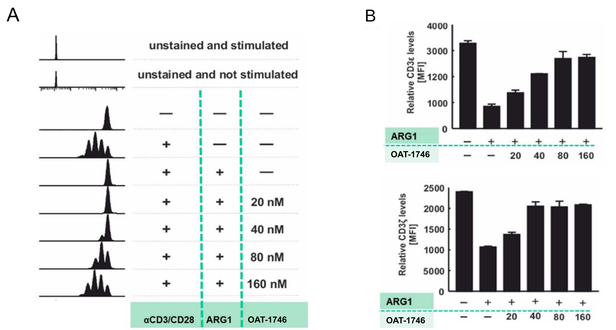

Fig. 1. *Restoration of T cell proliferation (A) and CD3 expression (B) of ARG1-suppressed T cells. Murine T cells were stained with cell trace violet and incubated with anti-CD3/CD28 beads to trigger proliferation. Recombinant hARG1 (250 ng/mL) and increasing concentrations (20-160 nM) of OAT-1746 were added. CD3ε and CD3ζ levels were measured using fluorochrome-conjugated antibodies by flow cytometry*

► OAT-1746 demonstrated strong efficacy as a monotherapy in 3 syngeneic tumor models.

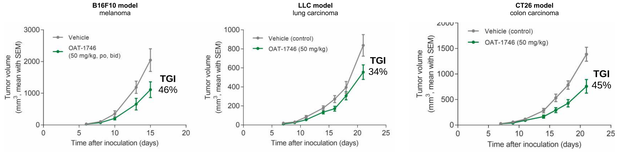

Fig. 2. *Effects of OAT-1746 on growth on B16F10, LLC and CT26 tumors. B16F10 and LLC cells were implanted s.c. in C57BL/6 and CT26 in BALB/c mice. OAT-1746 was dosed at 50 mg/kg, po, bid.*

ACKNOWLEDGEMENTS

These studies were supported by the project DIMUNO: „Development of new cancer therapies based on selective antitumor immunomodulators" – co-financed by the National Centre for Research and Development in the framework of STRATEGMED-3: Prevention practices and treatment of civilisation diseases.

Narodowe Centrum Badań i Rozwoju

STRATEGMED

► OAT-1746 showed a dose-dependent anti-tumor efficacy, which correlated with plasma concentration of the drug and significantly increased arginine levels in plasma which were sustained for 24 h (*Fig. 3*).

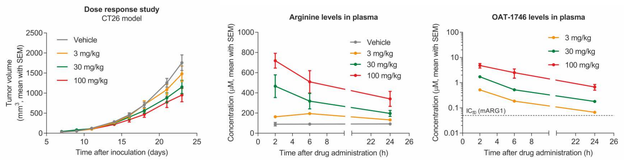

Fig. 3. *Dose-dependent anti-tumor efficacy of OAT-1746 in the CT26 model. CT26 cells were injected s.c. in BALB/c mice. OAT-1746 was dosed po bid as indicated. OAT-1746 and arginine levels were determined at the indicated time points after the last dose.*

► Increased efficacy of OAT-1746 in combination with gemcitabine and anti-PD-L1 antibody vs. monotherapy. Combinatorial immunotherapy of OAT-1746 and anti-PD-L1 antibody resulted in a "controlled" tumor growth with 55% of tumors remaining under 500 mm^3 at day 24.

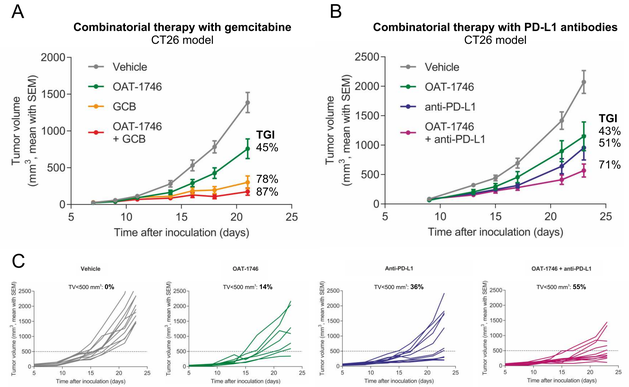

Fig. 4. *Tumor growth kinetics after OAT-1746 treatment (50 mg/kg, po, bid) alone or in combination with (A) gemcitabine (10 mg/kg, ip, 2 doses) or (B) anti-PD-L1 antibody (2.5 mg/kg, ip, 4 doses) in the CT26 model. Growth on individual tumors shown in (C).*

► OAT-1746 exhibited a strong PD effect resulting in 4-7 fold increase of arginine levels in plasma and tumors (*Fig. 5*). The arginine plasma levels (400-600 µM) exceeded several fold the arginine concentration required for the maximal stimulation of T cell proliferation (120 µM) determined in *ex vivo* assays.

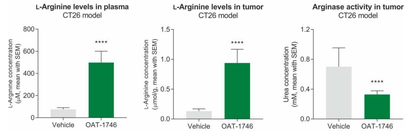

Fig. 5. *PD effects of OAT-1746 in CT26 tumor model. OAT-1746 was dosed at 50 mg/kg bid po for 24 days. Arginine levels in plasma and tumors were determined 2h after the last dose. Arginase activity was measured in tumor 2h after the last dose.*

► OAT-1746 significantly increased the expression of markers of NK and T-cell activation in CT26 tumors indicating reactivation of the inflammatory tumor microenvironment and confirming anti-immunosuppressive activity.

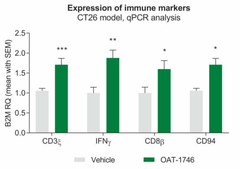

Fig. 7. *Induction of the T and NK cells markers in CT26 tumors by OAT-1746. OAT-1746 was dosed at 50 mg/kg bid ip for 14 days. Tumors were extracted, RNA was prepared and expression of immune markers was evaluated by rtPCR. Fold induction versus vehicle treated tumors was calculated.*

CONCLUSIONS

► OAT-1746 is highly active, selective dual inhibitor of ARG1 and ARG2 with a potent cellular activity.

► OAT-1746 showed good pharmacological properties and a significant anti-tumor efficacy in multiple tumor models as a monotherapy and in combinations including gemcitabine and anti-PD-L1 antibody with no aparent toxicity.

► OAT-1746 efficacy correlated with a PD effects including inhibition of tumor arginase activity and 4-7 fold increase in plasma and tumor arginine levels.

► Induction of markers of T and NK cell activation in tumor microenvironment confirmed the reversal of ARG-mediated immunosupression by OAT-1746.

► These results support the clinical development of OAT-1746 for cancer therapy.