

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

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BACKGROUND

Depletion of arginine represents an important mechanism of immunosuppression and high plasma and tumor arginase (ARG) activity have been demonstrated in patients with a wide spectrum of cancers and correlated with a poor prognosis. Low arginine levels inhibit proliferation and activation of cytotoxic T and NK cells. Preclinical and clinical studies confirmed that simultaneous interference with multiple mechanisms of immunosuppression resulted in a strongly improved antitumor efficacy. In this context, we have developed OAT-1746 - a novel, potent and selective small molecule inhibitor of ARG1 and ARG2 and evaluated its antitumor efficacy as a monotherapy and in combinations with gemcitabine and inhibitors of PD-L1 and IDO.

METHODS

The IC₅₀ values 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. The *in vivo* antitumor efficacy was evaluated in syngeneic mouse models after oral administration. Quantitative real-time PCR was used to determine inflammatory markers. The tumor arginase activity was assessed using the urea detection assay. L-Arginine and drug levels in plasma and tumor were evaluated by LC/MS method.

RESULTS

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

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	Cell-based assays	IC ₅₀
hARG1	32 nM	M2 murine macrophages	55 nM
hARG2	75 nM		
mARG1	50 nM	ARG1-transfected CHO-K1 cells	34 nM
rARG1	73 nM		

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

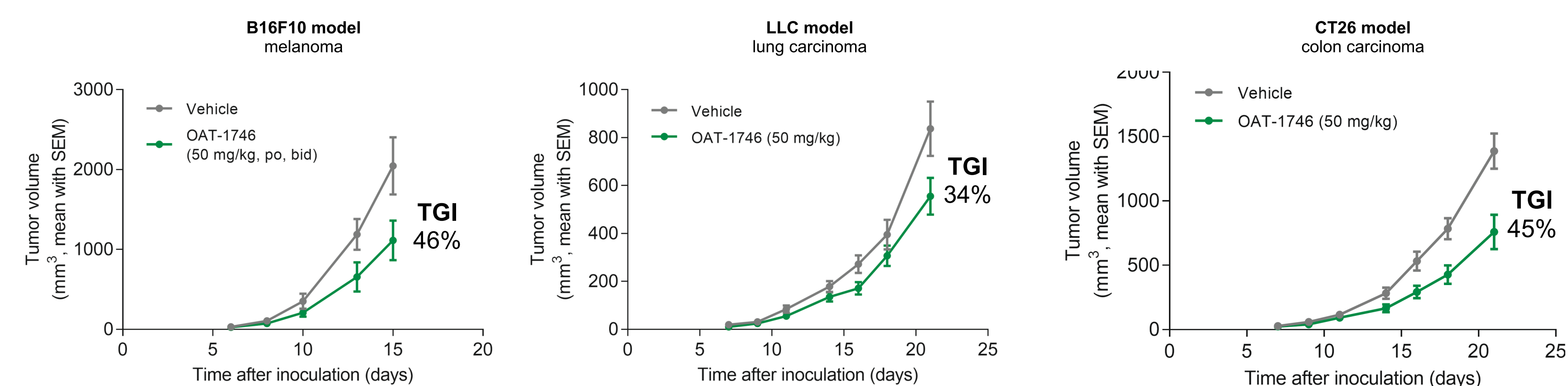


Fig. 1. Effects of OAT-1746 on growth of B16F10, LLC and CT26 tumors. B16F10 and LLC cells were implanted s.c. in C57BL/6 and CT26 in BALB/c mice. OAT-1746 was dosed at 50 mg/kg, po, bid.

► OAT-1746 exhibited a dose-dependent anti-tumor efficacy, which correlated with plasma drug concentration and significantly increased plasma arginine levels which were sustained for 24 h (Fig. 2).

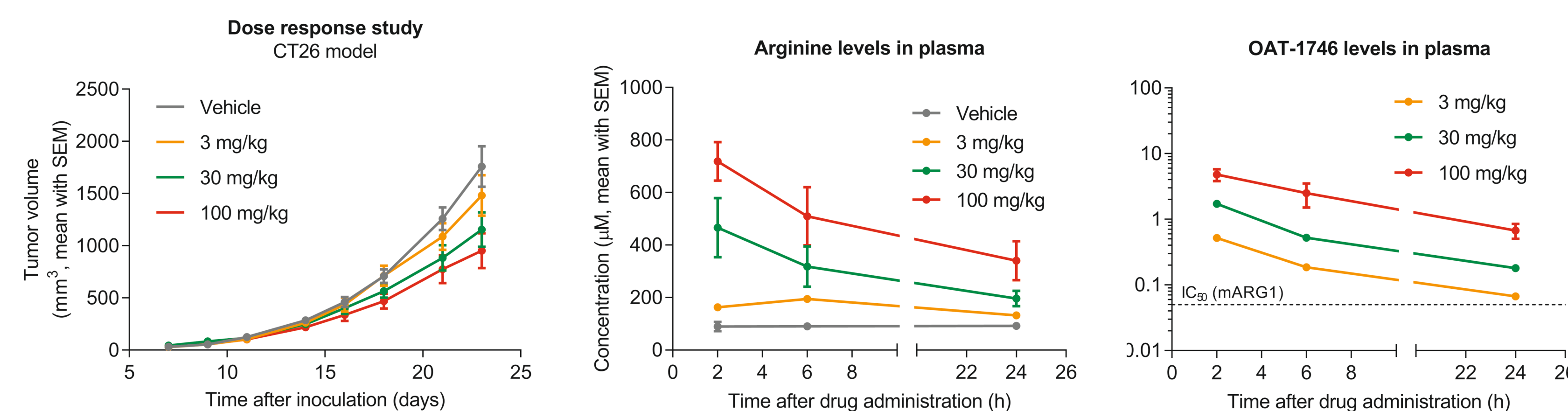


Fig. 2. Dose-dependent anti-tumor efficacy of OAT-1746 in the CT26 model. CT26 cells were injected s.c. in BALB/c mice. OAT-1746 was dosed po bid as indicated. OAT-1746 and arginine levels were determined at the indicated time points after the last dose.

► 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 mm³ at day 24 (Fig. 3).

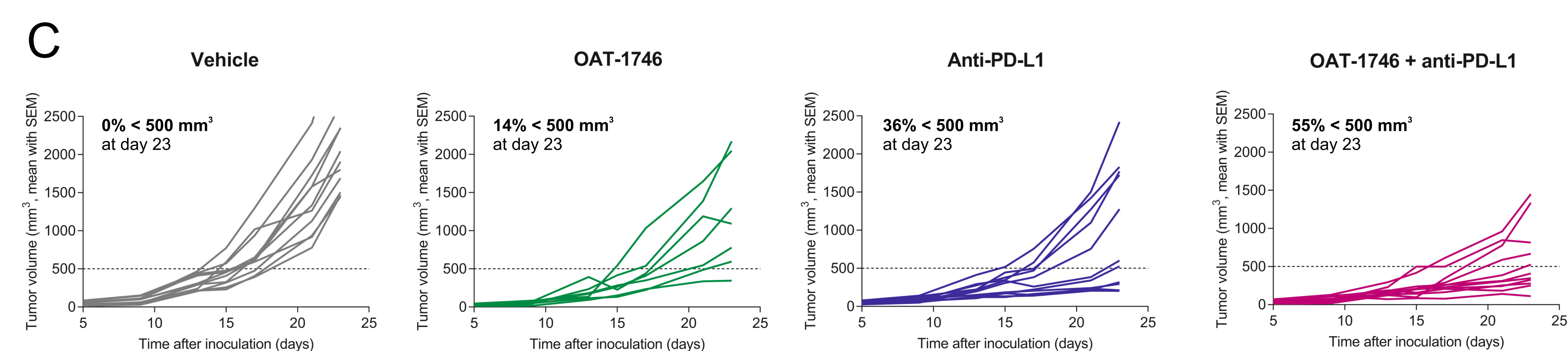
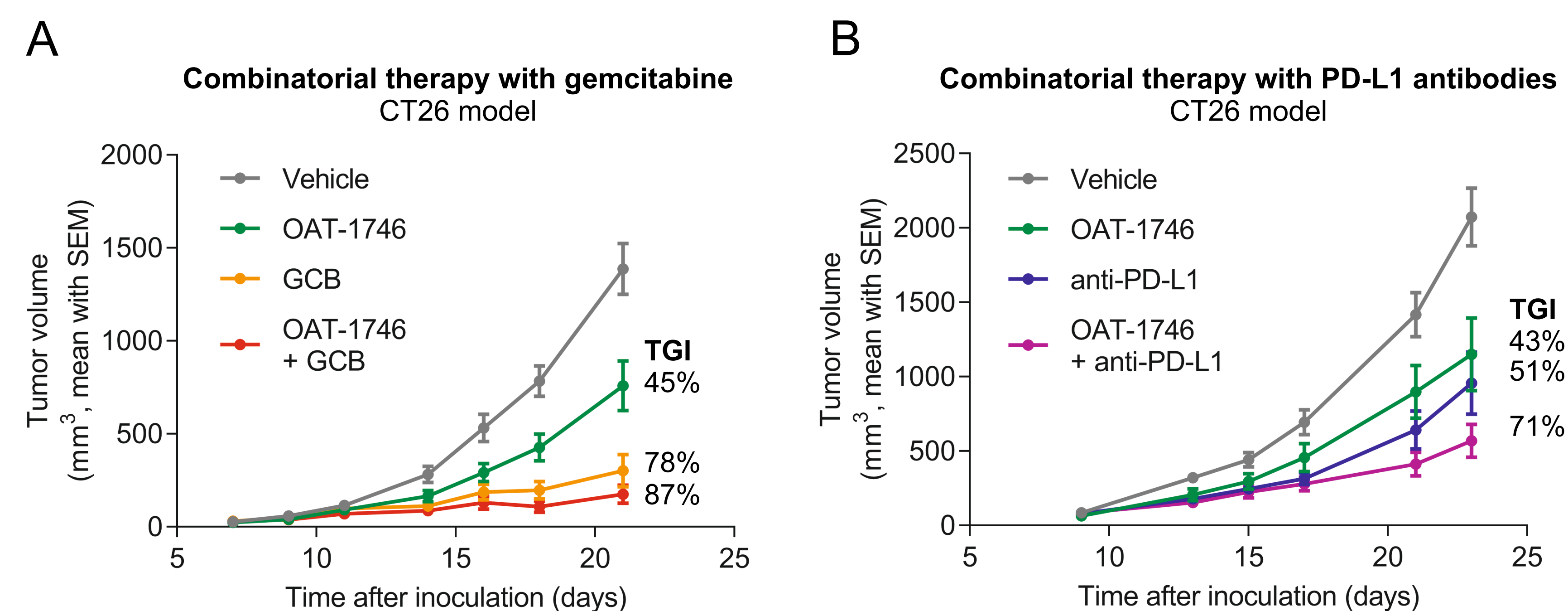


Fig. 3. Tumor growth kinetics after OAT-1746 treatment (50 mg/kg, po, bid) alone or in combination with (A) gemcitabine (50 mg/kg, ip, 2 doses) or (B) anti-PD-L1 antibody (2.5 mg/kg, ip, 4 doses) in the CT26 model. Growth of individual tumors shown in (C).

ACKNOWLEDGEMENTS

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► OAT-1746 significantly increased the antitumor efficacy of the PD-L1 + IDO inhibitor dual immunotherapy in the CT26 model. Rechallenge of the mice which completed the triple immunotherapy (OAT-1746 + PD-L1 + IDO) with CT26 cells resulted in a strongly suppressed tumor growth suggesting development of the antitumor immunity (Fig. 4).

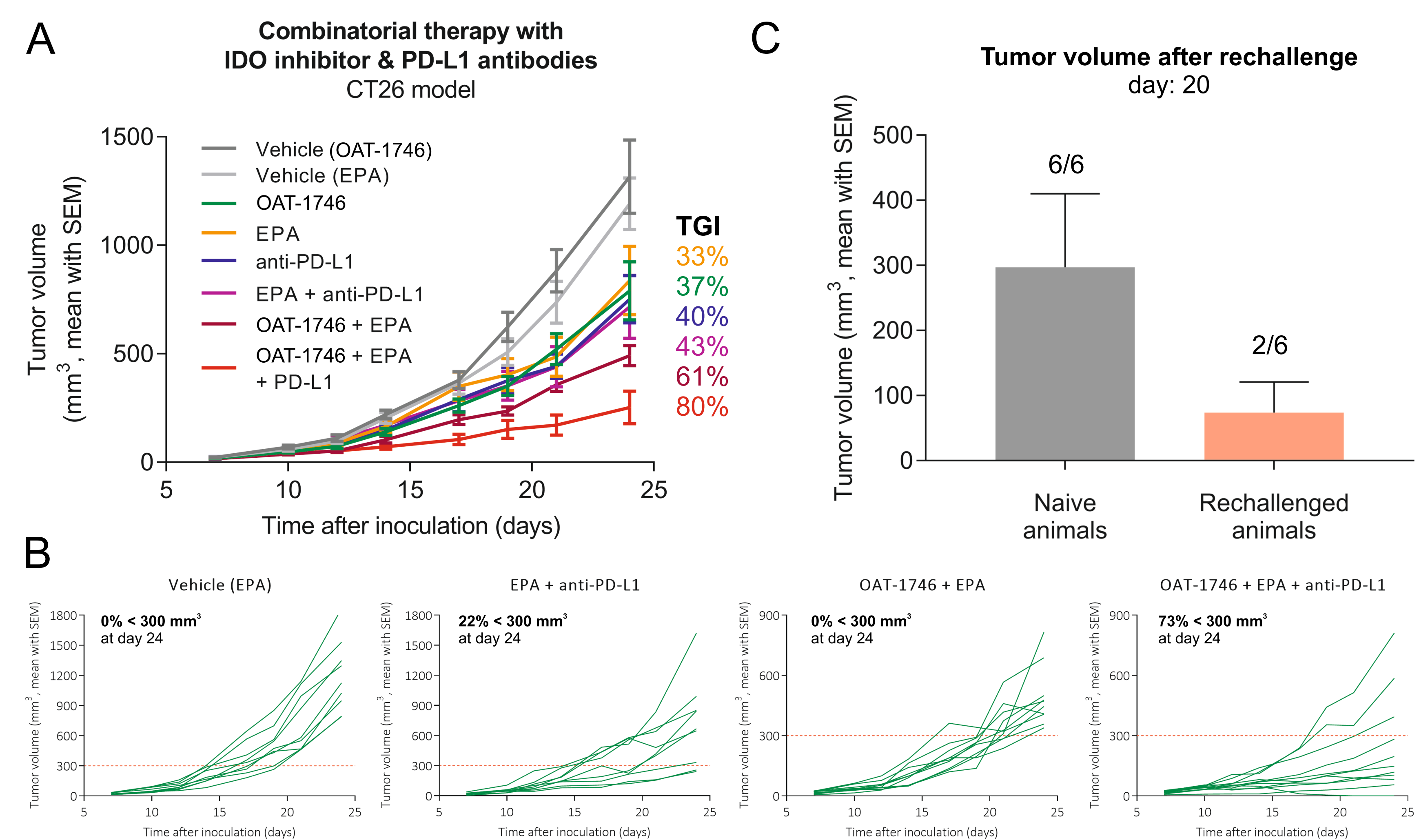


Fig. 4. Effects of OAT-1746 (50 mg/kg, po, bid) in combination with epacadostat (30 mg/kg, po, bid) and anti-PD-L1 (2.5 mg/kg, ip, 5 doses) on the tumor growth in the CT26 model (A, B). Following the triple therapy mice were rechallenged with CT26 cells injected into the contralateral side and the tumor growth was followed for 20 days and compared to the growth of tumors in naive animals (C).

► ARG1 expression is elevated in human gliomas and correlates with the disease stage. OAT-1746 effectively crossed the BBB and increased arginine levels in brains of rats bearing the C6 glioblastoma.

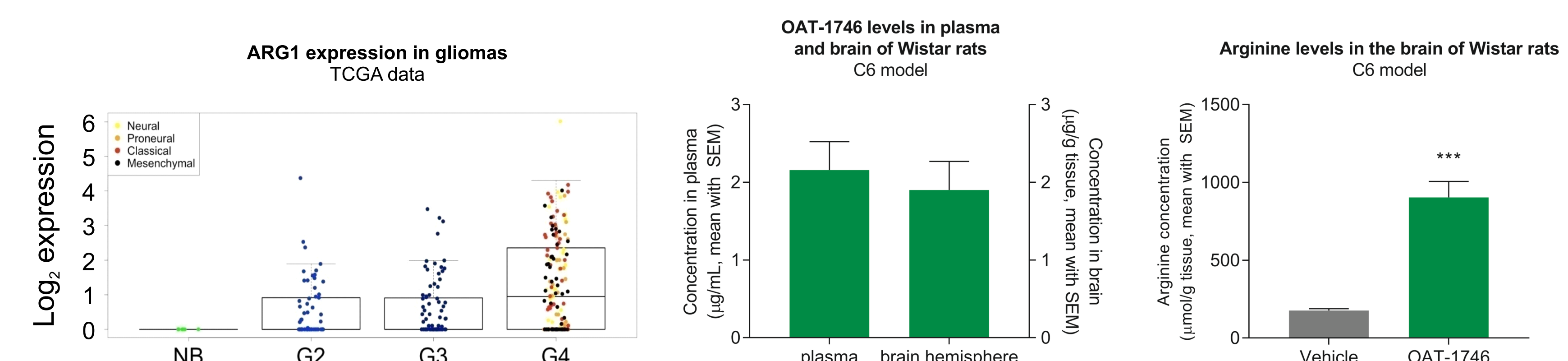


Fig. 5. Expression of ARG1 in human gliomas (data from TCGA). Brain OAT-1746 and arginine levels in the ipsilateral hemispheres of rats stereotactically injected with C6 GBM cells were analyzed after 20 days of dosing (25mg/kg, qd, po) 2h after the last dose.

► OAT-1746 exhibited a strong PD effect resulting in 4-7 fold increase of arginine levels in plasma and tumors (Fig. 6). The arginine plasma levels (400-700 µM) exceeded several fold the arginine concentration required for the maximal stimulation of T cell proliferation (120 µM) determined in *ex vivo* assays.

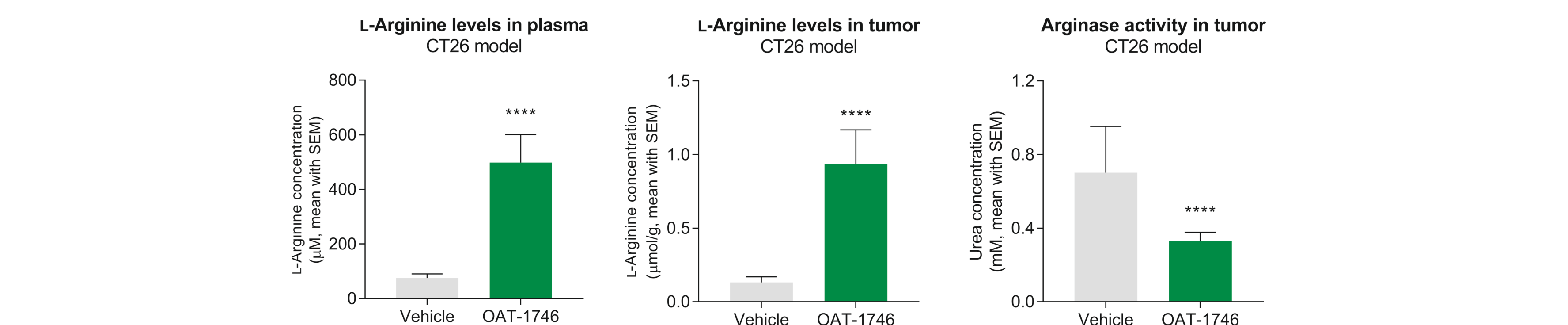


Fig. 6. PD effects of OAT-1746 in CT26 tumor model. OAT-1746 was dosed at 50 mg/kg bid po for 24 days. Arginine levels in plasma and tumor extracts were determined 2h after the last dose. Arginase activity was measured in tumor extracts 2h after the last dose.

► 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 its anti-immunosuppressive activity (Fig. 7).

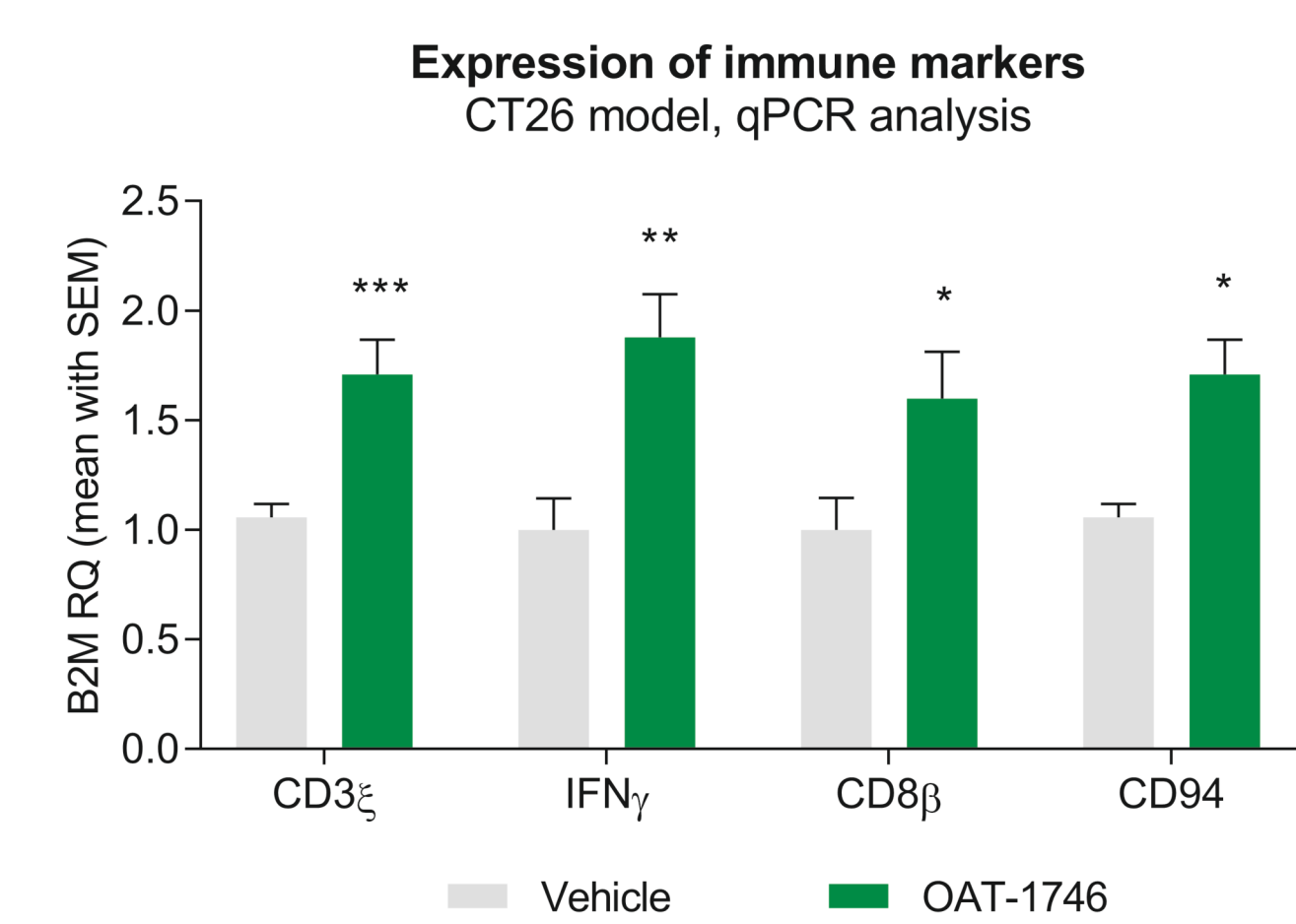


Fig. 7. Induction of the T and NK cells markers in CT26 tumors by OAT-1746. OAT-1746 was dosed at 50 mg/kg bid ip for 14 days. Tumors were extracted, RNA was prepared and expression of immune markers was evaluated by rtPCR. Fold induction versus vehicle treated tumors was calculated.

CONCLUSIONS

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

► OAT-1746 exhibited good pharmacological properties and a significant anti-tumor efficacy in multiple tumor models as a monotherapy and in combinations.

► OAT-1746 significantly increased antitumor activity of immune checkpoint inhibitors of PD-L1 and IDO, with the triple immune therapy demonstrating the best efficacy.

► OAT-1746 efficacy correlated with 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 immunosuppression by OAT-1746.

► Based on these results, OAT-1746 was nominated for a clinical development as OATD-02.