OBI-3424, an AKR1C3-activated prodrug, exhibits *in vivo* synergistic anti-tumor effect in combination with pembrolizumab by induction of immunogenic cell death

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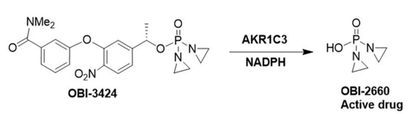
Background

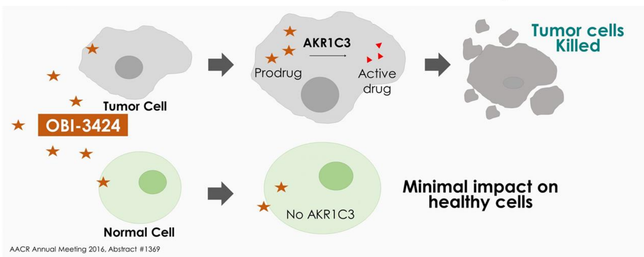
OBI-3424 is a prodrug of nitrogen mustard that is selectively cleaved by AKR1C3 to release an active, cytotoxic aziridine, which forms DNA crosslinks and cause cell death. Immunogenic cell death (ICD) involves the activation of cytotoxic T lymphocyte-driven adaptive immunity with long-term immunological memory. This study aims to investigate whether OBI-3424 can induce ICD and create a tumor microenvironment that benefits the combination therapy of OBI-3424 with immune checkpoint inhibitors. OBI-3424 is currently in Phase 1/2 clinical trials for solid tumor and acute lymphoblastic leukemia (NCT03592264 and NCT04315324).

Methods

OBI-3424 induced ICD was examined in vitro by incubation of the prodrug with AKR1C3 positive cells followed by the detection of damaging-associated molecular patterns (DAMPs). The ICD-related immunity was assessed in vivo using advanced severe immunodeficient mice that were engrafted with human peripheral blood mononuclear cells (PBMCs). Anti-tumor effect of OBI-3424 in combination with pembrolizumab was evaluated in a xenograft model using PBMC-humanized mice.

Structure and Mechanism of action of OBI-3424



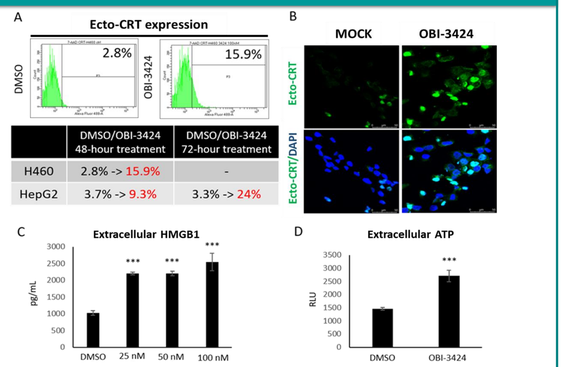


OBI-3424 is a chemically synthesized potent nitrogen mustard, which is selectively cleaved to the cytotoxic aziridine (OBI-2660) by AKR1C3 in the presence of NADPH. The active molecule OBI-2660 released by OBI-3424 is similar to the chemotherapeutic drugs thiotepa and mitomycin C, which leads to alkylation and cross-linking of DNA at the N7 (or O6) position of guanine.

Results

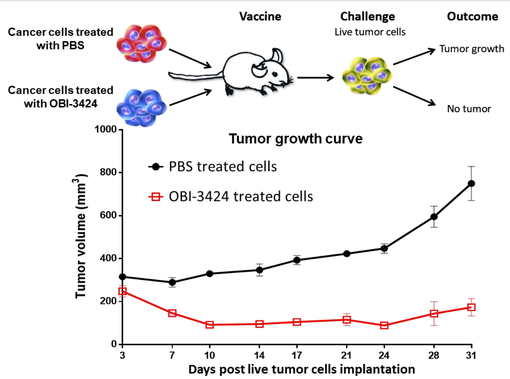
Incubation of OBI-3424 with AKR1C3 positive cells H460 and HepG2 induced the release of DAMPs including calreticulin, HMGB1, and ATP, in dose- and time-dependent manners (Fig.01). The detection of the DAMPs indicated that OBI-3424 induced ICD *in vitro*. The OBI-3424-induced ICD and its related immunity were also assessed *in vivo*. PBMC-humanized mice were immunized with OBI-3424- or PBS-treated HepG2 cells and then challenged with live HepG2 cells. No tumor growth was noted in mice that were immunized with OBI-3424-treated cells, indicating that the dying HepG2 cells induced by OBI-3424 elicited an adaptive, tumor-specific immune response (Fig.02). Furthermore, OBI-3424 showed a synergistic anti-tumor effect in combination with pembrolizumab in HepG2 xenograft model using PBMC-humanized mice. OBI-3424 plus pembrolizumab exhibited significantly stronger inhibition on tumor growth (TGI 77.2%) when compared with the treatment of OBI-3424 (TGI 27.8%) or pembrolizumab (TGI -15.3%) alone (Fig.03). Moreover, the combo benefits were totally diminished when CD8 T cells were excluded from the PBMC, which indicated that OBI-3424 treatment could further activate CD8 T cells to attack tumors and the combo benefits were CD8 T cell dependents. Analysis of tumor-infiltrating lymphocytes (TILs) showed that OBI-3424 treatment induced the populations of activated cytotoxic CD8 T-cells (CD45+/CD8+/CD69+ and CD45+/CD8+/Granzyme), activated helper CD4 T-cells (CD45+/CD4+/CD69+), and mature dendritic cells (CD11b+/CD86+) (Fig.05). In addition, OBI-3424 treatment also increased PD-1 expression on CD8 and CD4 cells, which in turn potentiated the anti-tumor effect of pembrolizumab (Fig.05).

Fig.01. OBI-3424 elicits DAMP molecules in vitro



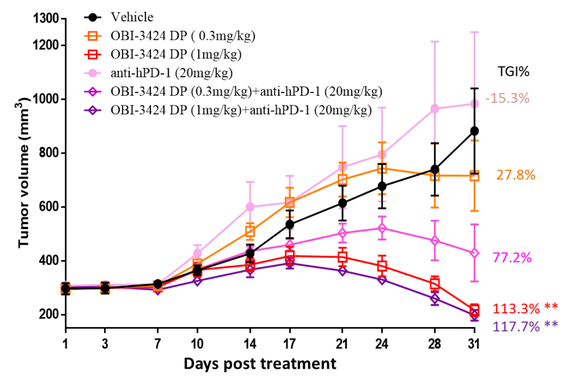
OBI-3424 elicits ICD in vitro. (A) Ecto-calreticulin (CRT) detected by flow cytometry on H460 and HepG2 after treatment with 100nM OBI-3424 for 48 or 72 hours. (B) Immunofluorescent staining of CRT (green) in H460 treated with100nM OBI-3424. Blue, nuclei. Scale bar, 50 µm. (C) ELISA of HMGB1 in the culture supernatant from H460 treated with OBI-3424 25, 50 and 100nM. (D) Extracellular ATP was measured in the culture supernatant after H460 cells were treated with OBI-3424 100nM. Data represent mean ± S.D. *p < 0.05, **p < 0.01, ***p < 0.001. Statistical analysis: Student's t-test

Fig.02. OBI-3424 treated HepG2 cell vaccine prevented tumor formation in vivo



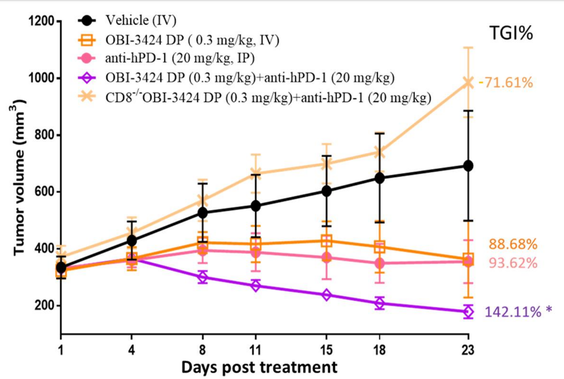
Vaccination of OBI-3424-treated HepG2 cells prevented tumor growth in vivo. HepG2 cells vaccine were prepared by treating cancer cells with PBS or OBI-3424 (3 µM) for 96 hours. The treated cells were implanted subcutaneously into the left flanks of advanced immunodeficient mice that were reconstituted with human PBMC one day earlier. Seven days later, repeated the implantation of the treated cells. Fourteen days later, live and untreated HepG2 cells were implanted subcutaneously into the right flanks of the mice.

Fig.03. Combination of OBI-3424 with Pembrolizumab exhibits strong additive efficacy in HepG2 humanized mice model



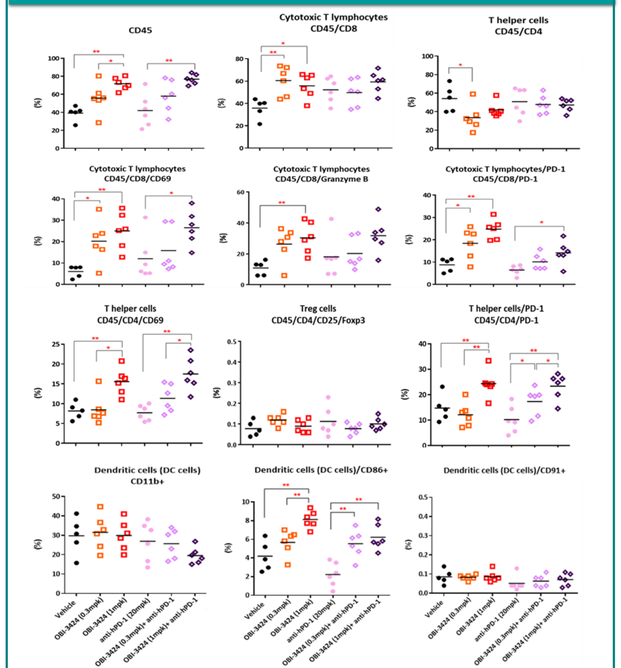
OBI-3424 was administered intravenously once weekly for 4 weeks.. Anti-PD1 antibody (pembrolizumab) was given intraperitoneally twice weekly for 4 weeks. Each group consisted of 6 human PBMC reconstituted HepG2 tumor-bearing mice. Data points represent group mean tumor volume ± standard error of mean (SEM). Statistically significant differences between groups were analyzed using student t-test. P*<0.05, P**<0.01, P***<0.001.

Fig.04. Combination of OBI-3424+Pemprolizumab elicits CD8+ T cell dependent antitumor immunity



OBI-3424 was administered intravenously once weekly for 2 weeks. Anti-PD1 antibody (pembrolizumab) was given intraperitoneally twice weekly for 2 weeks. CD8 T cells were depleted by magnetic beads before injected into mice in CD8-/- group. Each group consisted of 6 human PBMC reconstituted HepG2 tumor-bearing mice. Data points represent group mean tumor volume ± standard error of mean (SEM). Statistically significant differences between groups were analyzed using student t-test. P*<0.05, P**<0.01, P***<0.001.

Fig.05. Immune cells infiltration and activation under the treatment of OBI-3424 ± anti-PD-1



Tumor infiltrated lymphocytes (TILs) analysis was performed at the end of in vivo study (Fig.03). HepG2 tumors of all groups were collected, and the digested primary cells from the tumors were stained for corresponding immune cell markers and analyzed by flow cytometer. Each group consisted of 6 human PBMC reconstituted HepG2 tumor-bearing mice. Each dot represents of one individual mouse. Statistically significant differences between groups were analyzed using student t-test. P*<0.05, P**<0.01, P***<0.001.

Conclusion

We demonstrated that OBI-3424 was able to induce ICD as shown by the release of DAMPs in vitro and tumor-specific immunity in vivo. OBI-3424 also created a tumor microenvironment that enhances the function of pembrolizumab, supported by the synergistic effect in animals with the cotreatment of the two drugs. The results suggest that a combination therapy of OBI-3424 and anti-PD-1 in human clinical study is warranted. OBI-3424 is currently in Phase 1/2 clinical trials for solid tumor and acute lymphoblastic leukemia (NCT03592264 and NCT04315324).

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