Discovery and optimization of potent and selective inhibitors of USP7 to enhance anti-tumor immunity and target tumor growth

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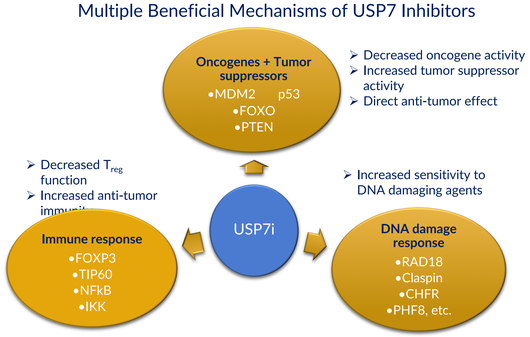
Abstract

USP7 is a deubiquitinase (DUB) that regulates the levels of multiple proteins with roles in cancer progression and immune response. USP7 has been reported to stabilize oncogenes such as MDM2, destabilize and inactivate the tumor suppressors p53 and PTEN, impart resistance to DNA-damaging chemotherapy by stabilizing proteins involved in DNA damage responses, and enhance the suppressive function of regulatory T cells (T_{reg}) by stabilizing the transcription factor FOXP3. We have designed USP7 inhibitors (USP7i) that are highly potent in biochemical and cellular assays and selective for USP7 over all other DUBs. Our inhibitors bind USP7 and reduce viability of the MM.1S tumor cell line, relieve mouse T_{reg} suppression of $CD8^+$ cell proliferation in vitro, and enhance inflammation in the contact hypersensitivity model *in vivo*. In addition, our compounds are orally bioavailable with moderate permeability and low clearance.

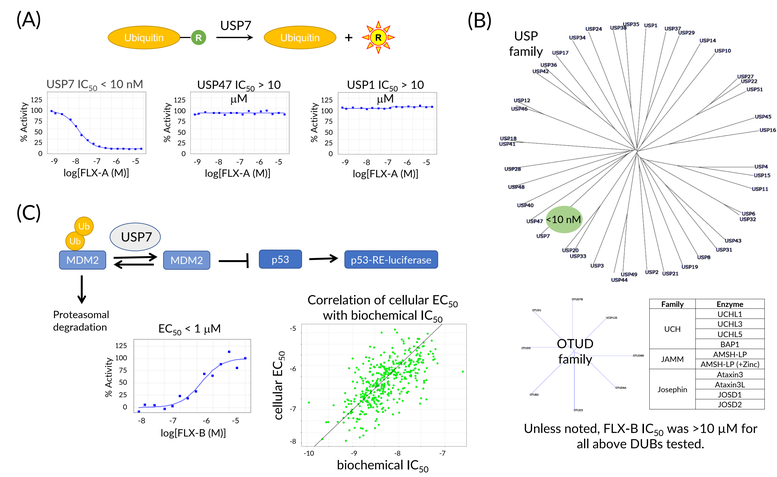
Multiple Beneficial Mechanisms of USP7 Inhibitors



Biochemical Potency of Compounds Used in These Studies

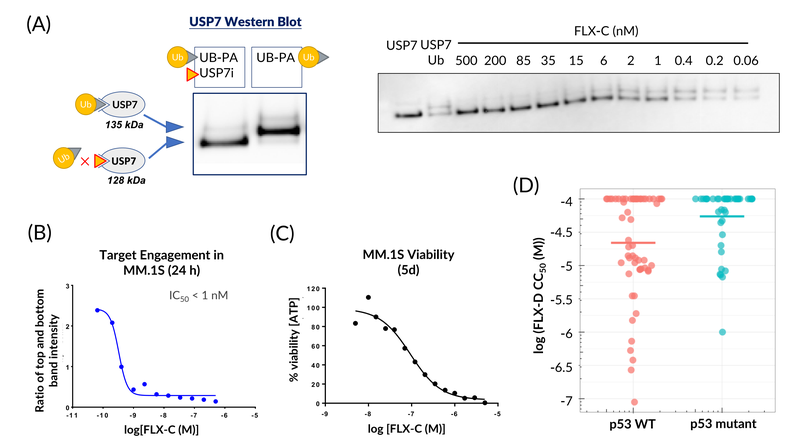
USP7i	USP7 IC_{50}
FLX-A	< 10 nM
FLX-B	< 10 nM
FLX-C	< 1 nM
FLX-D	< 10 nM
FLX-E	< 1 nM

1 Novel USP7 Inhibitors are Highly Selective and Potent in Biochemical and Cellular Assays



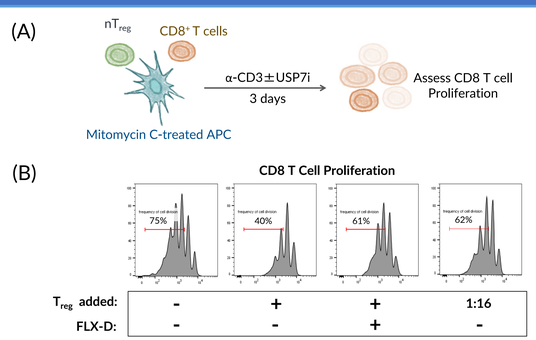
(**A**) Biochemical activity of FLX USP7 inhibitor (USP7i) against USP7 and selectivity over USP47 and USP1 in an assay using rhodamine-labeled ubiquitin. (**B**) Selectivity of FLX USP7i over all other DUBs. (**C**) Cellular activity of FLX USP7i in a luciferase reporter gene assay of p53 activation.

2 Novel USP7 Inhibitors Show Target Engagement and Tumor Cell Line Cytotoxicity Predicted by p53 Status



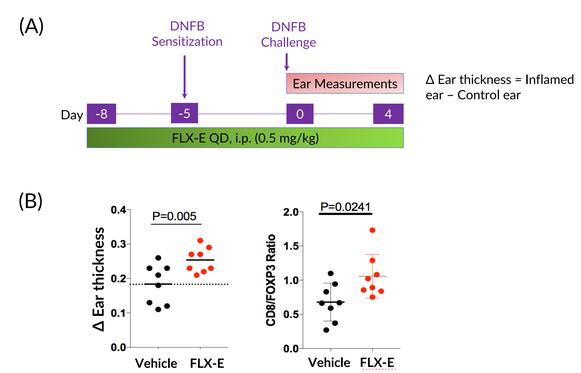
(**A**) Schematic of target engagement assay and a representative Western blot. Treatment of MM.1S cells with FLX USP7i results in target engagement as measured at 24 hr (**B**) and dose-dependent cytotoxicity in a 5-day assay (**C**). (**D**) Analysis of mutational predictors of sensitivity of a panel of 100 cell lines to FLX USP7i indicated that p53 status was an important predictor of sensitivity.

3 USP7 Inhibitors Relieve Mouse T_{reg} Suppression of $CD8^+$ T Cell Proliferation



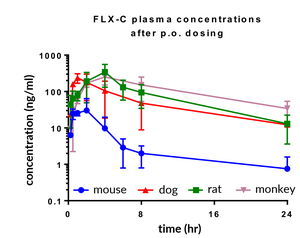
(**A**) $CD8^+$ T cells were co-cultured with natural T_{reg} cells and antigen-presenting cells in the presence of FLX USP7i for 4 days, after which $CD8^+$ cell proliferation was assessed by flow cytometry. (**B**) FLX USP7i reverses suppression of $CD8^+$ T cell proliferation by ~40% (equivalent to diluting T_{reg} by 16-fold).

4 USP7 Inhibitors Enhance Inflammation in Contact Hypersensitivity Model *in Vivo*



(**A**) Schematic of the contact hypersensitivity model: mice are sensitized to and later challenged with a hapten, DNFB (1-fluoro-2,4-dinitrobenzene). T_{reg} function in both sensitization and challenge phases. (**B**) FLX USP7i increases inflammation as measured by changes in ear thickness and in CD8/FOXP3 ratio.

5 USP7 Inhibitors are Highly Bioavailable



	mouse	rat	dog	monkey
% F	6	12	62	88

FLX USP7i have desirable bioavailability, allowing the possibility of oral dosing in additional pharmacological models.

Conclusions

- We have developed a novel series of potent, highly selective small molecule USP7 inhibitors with activity both *in vitro* and *in vivo*.
- FLX USP7i activate p53 in cell-based assays and are cytotoxic to the MM.1S tumor cell line *in vitro*.
- FLX USP7i increase inflammation and change CD8/FOXP3 ratio *in vivo*, indicating immune activation.
- **We believe our USP7 inhibitors are novel immunomodulators with multiple beneficial mechanisms of action including direct killing of tumor cells.**