AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

In order to ensure that all of the key resources used in this research project are authenticated and remain consistent and valid throughout the funding period, we propose the following plans for the cell line, antibodies, chemicals and mice, illustrated with reagents that will be used most frequently.

Cell lines: A549 [ATCC CCL-185]. This is a primary tumor cell line that will be used throughout this project. To ensure authentication and consistency throughout the funding proposal, the cell line will be tested using short tandem repeat profiling and only low passage cells will be used in the experiments outlined in this proposal. Passage 5 following arrival from ATCC will be used for all experiments. This will be accomplished by the following: (**1**) A549 from ATCC will be expanded following the ATCC growth protocol. Aliquots will be cryopreserved starting at passage 3. (**2**) After passage 5, 50-100 aliquots will be cryopreserved.   Passage 5 aliquots will be used for all A549 experiments outlined in the proposal. (**3**) An aliquot of passage 5 will be sent for cell line authentication using short tandem repeat profiling at Idexx BioResearch. (**4**) Once passage 5 aliquots are depleted, a low passage vial will be expanded to replenish the passage 5 stocks and sent to Idexx BioResearch for authentication. We anticipate having to do this every 2 years. (**5**) Once our low passage stocks are depleted we will purchase another aliquot from ATCC and follow the same process.

Antibodies: Two key antibodies will be used in this application for *in vivo* neutralization studies: (**a**) anti-CD8 (clone YTS.169.4) purchased from BioXcell, and (**b**) anti-PD1 (clone 4H2) from Bristol Myers Squibb.

To ensure authentication as well as consistent and reproducible results for these antibodies, they were obtained in bulk and are stored in 1-2mg/ml aliquots at -80°C. Upon thawing, the antibodies will not be re-frozen and used within 1 month. Antibody specificity for the two clones will be verified by flow cytometry. New batches of mAb will also be tested side-by-side with older batches to ensure lot-to-lot consistency. Finally, isotype control mAbs will be verified using a standard isotyping kit.

Pharmaceuticals: For the two key pharmaceuticals used in the proposal, we will authenticate by using strict controls in each experiment. AZD6738, 50 grams was obtained from AstraZeneca, and VX-970 (S7102) was purchased from Selleckchem.

To ensure authentication as well as consistent and reproducible results for these pharmaceuticals, AZD6738 will be stored as a powder in 1 gram aliquots. For mouse experiments, AZD6738 is prepared for oral gavage within one hour of use. VX-970 will be purchased in bulk and stored in powered form. For use in tissue culture, AZD6738 and VX970 will be dissolved in DMSO and stored in aliquots at -20°C for less than 6 months. These solutions will not be refrozen after use. New batches of pharmaceuticals will also be tested side-by-side with older batches to ensure lot-to-lot consistency.

We will validate findings with AZD6738 with a second clinical ATRi, VX-970 which has an unrelated structure. To validate ATRi target engagement, we will immunoblot phosphorylated ATM, Chk1, and Chk2. To validate that findings associated with ATRi are caused by ATR kinase inhibition, we will use DLD-1 cells harboring a homozygous ATR mutation, as well as siRNA to disrupt the expression of ATR, as we have done previously.

Mice: To ensure the authenticity of mice that will be used in this project, breeders will be genotyped for all of the targeted alleles in the strain using validated PCR protocols with wild type and relevant heterozygous and knock-in controls used for each genotyping procedure. All mice from non-homozygous breeding pairs will also be genotyped for confirmation prior to use in experiments. The genetic background of all mice are characterized by SNP analysis using the GoldenGate protocol and analyzed using the VeraCode bead system on the Illumina BeadXpress.