Authentication of Key Biological and/or Chemical Resources

Tumor models: For this project we will use two types of tumor models established in mice: 1) immortalized ovarian cancer cells derived from tumors originated in immunocompetent $Pten^{-/-}$ /$P53^{(R172H)}$ double-mutant C57BL/6 mice from which the murine ovarian cancer cell line MOC-1 was derived, 2) patients derived xenografts (PDXs) generated by our lab from patients that were sub-optimally debulked and that present activation of the TGF-β signaling. All our models have been confirmed for the Müllerian epithelial origin (PAX8 and Cytokeratin 7 positive), genetic identity (through DNA fingerprint and NGS based CNV analysis) and tumorigenicity in mice.

We do not expect significant changes in these models throughout the short period of this award as we will be using frozen, low passage aliquots of the murine cell line and frozen, single use only, PDX samples collected and tested after the first growth passage in mice. However, our models can be authenticated through immunohistochemistry analysis of PAX8 and Cytokeratin 7 and CNV analysis.

We will use only commercially available mice purchased from Charles River laboratories. These include: C57 black 6 mice to grow the murine tumor model, and SCID SHO mice to grow the PDXs.

Immunohistochemistry: For the immunohistochemistry analysis we will use commercially available antibodies. These include: 1) the antibody targeting the phosphorylation marker pSmad2/3 (as a surrogate marker of TGF-β pathway activation) that was used in our preliminary results (Cell Signaling), and 2) additional antibodies that will be used as determined from the results of the experiments described in Aim 1.

Gene expression (NanoString) analysis: we will use customized probes designed and produced by NanoString Technologies for each gene of the debulking signature that will be validated in Aim 1.

TGF-β inhibition Systematic TGF-β targeting in xenograft bearing mice will be achieved by LY2157299 (Eli Lilly& Co.) and Fresolimumab (Genzyme). LY2157299 is a small molecule inhibitor of TGF-β receptor I (TGFBR1). Fresolimumab is a humanized antibody neutralizing all three TGF-β ligands. Both these drugs have been used in clinical trials for other cancers and will be provided by the respective companies. For adaptation into murine system, an equivalent clone **1D11** which is capable of sequestering TGF-β1/2/3 from both human and murine origin will be used herein and provided by Genzyme.

TGF-β will be also inhibited by editing the celsl through the CRISPR system including a plasmid expressing CAS9 to be used in combination with a Guide RNA plasmid. Both these plasmids are commercially available from AddGene. The TGF-β-specific oligos were designed in house and cloned into Guide RNA plasmid. The sequence of these oligos will be made public upon publishing the results.