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# Development and characterization of *in vitro* assays to quantitate and detect tubulysin B hydrazide in biological samples

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## Introduction

► EC1456 is a folate-targeted small molecule drug conjugate (SMDC) of tubulysin B hydrazide (TubBH) that is currently in Phase 1 clinical trials for treatment of patients with folate receptor (FR)-positive tumors.

► The ability to detect, quantitate, and localize EC1456 in tissues and biological fluids is particularly important for the characterization of its biodistribution and pharmaco-dynamic properties.

► To achieve these goals, we have generated a rabbit polyclonal antibody against TubBH and assessed its potential utility in multiple assays.

## Methods

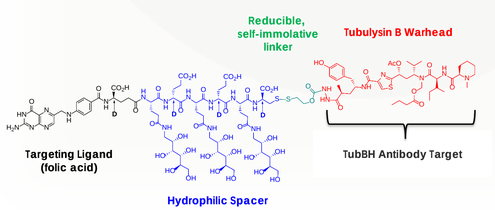
► A competitive ELISA was developed in which soluble TubBH competed for antibody binding to immobilized antigen.

► The ELISA was successfully used to determine intratumoral drug concentrations in FR-positive KB and FR-negative A549 xenograft tumor homogenates from animals dosed with either EC1456 or untargeted TubBH.

► An immunohistochemical method developed using the antibody allowed for visualization of drug uptake in KB and A549 tumors following an efficacious dose of EC1456.

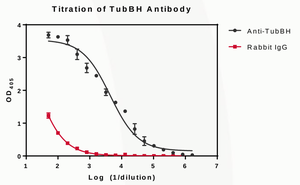
► The antibody was also successfully utilized to detect cell surface FR-bound EC1456 on fixed and unfixed KB cells *in vitro* by flow cytometry.

## Chemical structure of EC1456

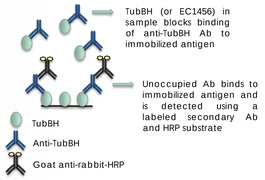



## Results

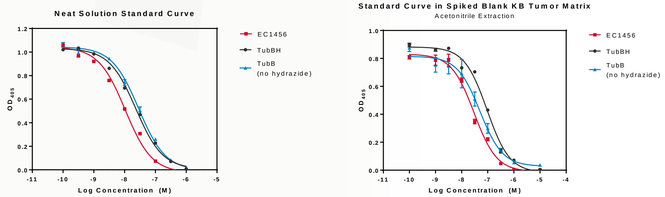
### Direct ELISA shows high affinity and specificity of the antibody for its target



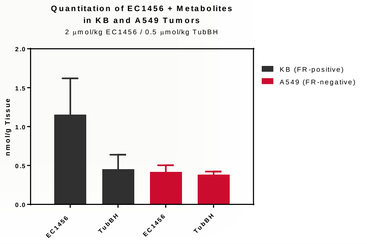

### Design of a competitive ELISA assay to quantitate TubBH



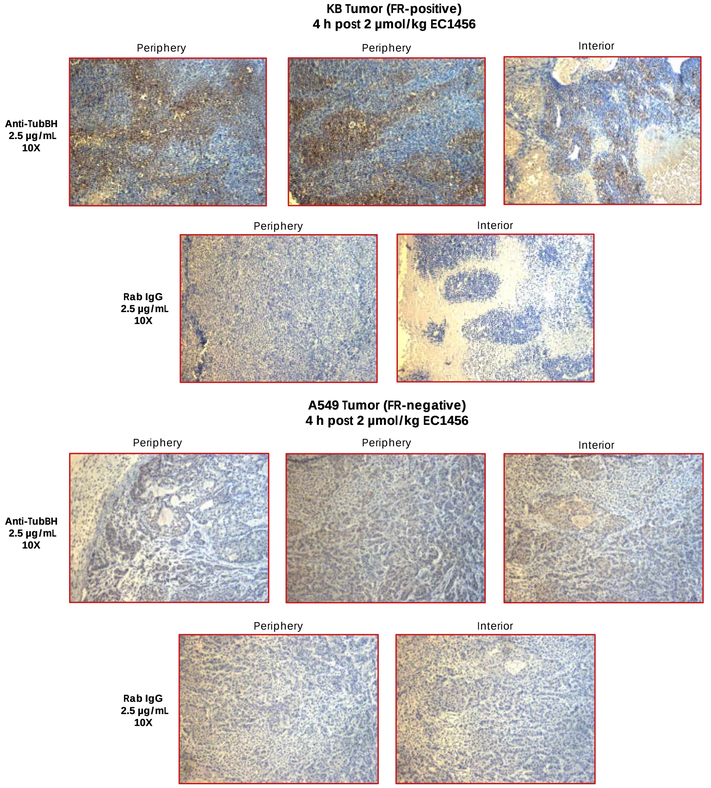

### Standard curves for EC1456 and associated metabolites in neat solution and spiked KB tumor homogenates



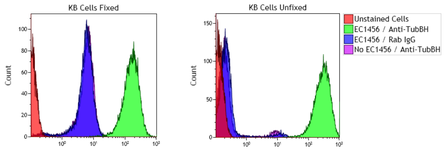

### Quantitation of EC1456 and associated metabolites in tumors from dosed animals




### Immunohistochemical analysis of EC1456 distribution in KB and A549 tumors




### *In vitro* detection of EC1456 on KB cells by flow cytometry




## Summary

► The antibody produced against TubBH allows for specific and sensitive detection of drug in biological samples utilizing a variety of methodologies.

- The antibody recognized conjugates of TubBH (ex. EC1456) as well as free TubBH (both with and without the hydrazide), which are known *in vivo* metabolites of the drug.

► A competitive ELISA was developed and used to measure levels of EC1456 and metabolites in tumors from mice dosed with the drug.

- EC1456 drug levels were higher in FR-positive KB tumors compared to FR-negative A549 tumors.
- Untargeted TubBH resulted in lower tumor concentrations of the drug compared to EC1456 in KB tumors.

► The antibody was successfully utilized to stain KB and A549 tumors for drug uptake following an efficacious dose of EC1456.

- KB tumors showed pockets of cell staining, often near vessels, while other areas of the tumor were negative.
- A549 tumors had considerably lower levels of drug in the tumor as assessed by IHC.

► The antibody was able to detect surface-bound EC1456 on both fixed and unfixed KB cells *in vitro*.

► Future work will determine the kinetics of drug retention in the tumor following dosing and analysis of clinical samples.