Quantitative Analysis of Cellular Processing of Antibody-Drug Conjugates

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ABSTRACT

Antibody-Drug Conjugates (ADCs) are a promising therapeutic class which combines the potency of chemotherapeutic drugs with the specificity of a tumor targeting antibody. ADCs aim to reduce systemic toxicity and maintain or improve therapeutic efficacy. Once an ADC reaches a tumor and binds its target antigen, it is internalized via receptor-mediated endocytosis. The ADC is internalized into an endosomal/lysosomal compartment, where the ADC is degraded, releasing the drug component from the antibody. The drug component can then leave the endosomal/lysosomal compartment and bind its intracellular target; hopefully, resulting in tumor cell killing.

In this thesis, we focus on how ADCs get processed at a cellular level. First, we developed a flow cytometric clonogenic assay and used this assay to study the single-cell potency of the chemotherapeutic drug doxorubicin. Across a number of cancer cell lines, we found that a cell’s ability to proliferate was only dependent on the amount of doxorubicin inside the cell and independent of varying drug media concentration, length of treatment time, or treatment with verapamil. We established a single-cell IC$_{50}$ of 4 – 12 million doxorubicin molecules per cell.

Next, we developed a model for ADC cellular processing and parameterized this model using a clinically approved ADC, T-DM1. Sensitivity analysis suggests that the amount of drug that is delivered to cells is a function of the amount of drug that comes in via internalization and the amount the leaves via drug efflux. This work also demonstrates how it is important to consider ADC processing as a complete system rather than isolating individual steps when designing ADCs. We also incorporated this cellular level processing model into a larger pharmacokinetic/pharmacodynamic model.

Finally, we used fluorescence microscopy with a Trastuzumab-Doxorubicin ADC to track where within a cell the drug component traffics once released from the antibody. We find that the ADC does not deliver a significant number of doxorubicin molecules to the nucleus, suggesting that escape from the lysosome limits the amount of drug that can be delivered to its target via an ADC. The ability to escape the lysosome should be considered when designing an ADC.

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