Understanding and Characterizing the Bystander Effect for Antibody Drug Conjugates

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Background: Once processed by the Antigen-positive (Ag+) cells, Antibody-drug conjugates (ADCs) can release cytotoxic drug molecules that can diffuse into the neighboring antigen-negative (Ag-) cells to induce their cytotoxicity. This additional efficacy of ADCs is known as the ‘bystander effect’. Although widely acknowledged, the rate and extent of the bystander killing is not quantitatively understood yet.

Objectives: The objectives of this research was to develop an in vitro PK-PD model linking the extracellular and intracellular concentrations of ADC to characterize the observed rate and extent of bystander effect in a coculture system of Ag+ and Ag- cells.

Methods: Trastuzumab-vc-MMAE, with an average DAR of 4 was synthesized and characterized. A coculture system was created with different HER2 expressing cell lines as Ag+ cells and GFP-transfected MCF7 cells as Ag- cells. Total cell count was assessed by MTT assay and GFP-MCF7 cell count was assessed by measuring the fluorescence. Analytical methods were developed to quantify total Trastuzumab, free MMAE and total MMAE concentrations in media and cell lysate. Cell viability experiments were performed in the presence of T-vc-MMAE in different cocultures and was mathematically characterized. Cellular disposition studies were performed in N87 and GFP-MCF7 cells, where a single cell disposition model was used to quantitatively characterize concentrations of different analytes of T-vc-MMAE.

Results: Cell viability experiments revealed that the extent of bystander effect increases with increasing fraction of Ag+ cells as well as increasing HER2 expression on Ag+ cells. A notable lag time was also observed prior to significant bystander killing. PD modeling analysis also suggested that the bystander effect of the ADC can dissipate over the period of time as the population of Ag+ cells decline. Cellular disposition studies revealed a significant binding of MMAE to tubulin resulting in retention inside the cell. Individual cellular PK models in two cell lines were then combined together to describe the disposition of T-vc-MMAE in a coculture system.

Conclusion: A novel single cell PK model for two cell lines was developed to characterize the bystander effect data. In future, the model will be integrated with a tumor disposition model (1) to characterize ADC exposures in a heterogeneous tumor.

References: