OPTIMISATION OF CONDITIONS FOR A STABILITY STUDY OF A CD38 INHIBITOR

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Research Project

This study was conducted to optimise conditions for several assays being used to produce a robust stability study of a CD38 inhibitor, Nuzumumab. From this, an extended shelf life drug can be introduced onto the market by Bath ASU.
These findings are extremely important for remote pharmaceutical manufacturers like Bath ASU. Extending shelf life of drugs increases time for shipping and administration of products. This in turn increases the scope of customers that the company can distribute to.
A set of both biological activity assays and SE-HPLC chromatography were performed and optimised as part of this project.

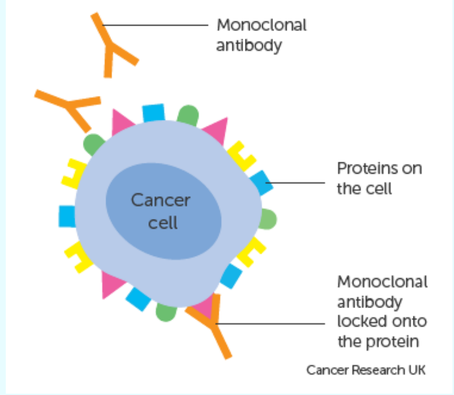
BATH ASU

Bath ASU is a pharmaceutical manufacturing compounder in Corsham, Wiltshire.
The company provides dose-banded and patient-specific chemotherapy, central intravenous additive products and patient-controlled analgesic products.

At **Bath ASU**, I was responsible for the production of batch documentation for the implementation of manufacture and release of products.

Other responsibilities included the handling of controlled drugs, releasing of batches and documentation, and involvement in quality improvement processes. I was also involved in compliance support activities and multiple administration duties.

NUZUMUMAB



Nuzumumab is a monoclonal antibody used in cases of relapsed multiple myeloma.
Multiple myeloma is a malignant cancer that occurs in the plasma cells of the bone marrow. Side effects arise due to build up of abnormal cells and increased levels of para-protein.
Nuzumumab mimics antibodies that naturally occur in the human body and are used by the immune system to aid in the fighting of infectious diseases.
Nuzumumab is an antagonistic inhibitor that causes cell lysis via multiple routes in the over-expression of the transmembrane glycoprotein, CD38.
Multiple myeloma remains incurable but Nuzumumab has been proven to potently inhibit CD38 and therefore decrease the level of side effects experienced by the patient.

Discussion

The biological activity assays carried out in the preliminary studies concluded that Nuzumumab does in fact trigger cell death by complement-dependent cytotoxicity and that optimal concentration of drug for this was 150 ng/ml.
Optimisation of SE-HPLC showed that peak symmetry and favourable chromatography characteristics improve with increasing concentration of Phosphate buffer. Maximal symmetry was determined to be 100 mM of this buffer.

Results

Biological Activity Assay

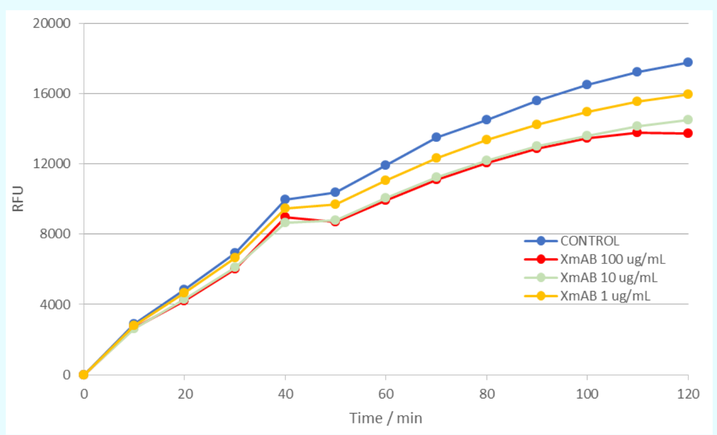


Figure 1: Biological activity of Nuzumumab with CD38 in a fluorescence measurement assay for increasing concentrations 1µg/ml, 10 µg/ml and 100 µg/ml. Inhibition is referenced against a positive control of Nuzumumab and CD38 alone in vitro.

An MTT assay with Nuzumumab showed that inhibition of CD38 increases with increasing concentration of the drug. However, maximal inhibition of 20% was reached at a value of 10 µg/ml and this extent of inhibition was not surpassed even with increasing concentrations of the drug in vitro.

At lower concentrations, inhibition is limited by availability of the drug in the body and hence maximal inhibition can not be reached as all target receptors would not be saturated. However, results seen at higher concentrations show the saturation of the target media with Nuzumumab.

Complement-dependent cytotoxicity assay

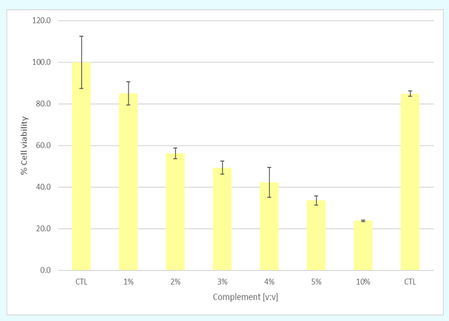


Figure 2: Viability of Daudi cells at varying baby rabbit serum concentrations determined using an MTT assay.

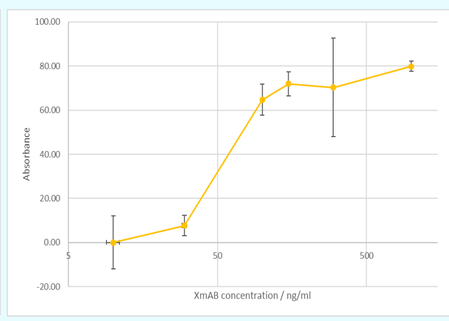


Figure 3: Absorbance reading of Resazurin assay at varying XmAB concentrations with relative standard deviations.

An Resazurin assay was completed using 6 sets of varying baby rabbit serum concentrations to determine the optimal concentration that would be used in the proceeding CDC assay experiments. It was seen that as serum concentration increases, degree of cell lysis significantly increases.

A serum concentration of 2% was chosen. This concentration was chosen as a compromise between 2 influential factors in the experiment. At high concentrations, excessive cell lysis for this experiment is seen where the reaction would no longer be Nuzumumab dependent. However, at low concentrations the limiting factor in the reaction would not be the mAB but the concentration of the serum. 2% serum was chosen as a middle ground where neither of these factors would become more influential than the other.

The relationship between Nuzumumab and degree of cell lysis showed a sigmoidal relationship. Inhibition increases with drug concentration however, a plateau in response is seen at 100 ng/ml, presumably where clearance of the drug from the receptors has reached capacity.

SE-HPLC optimisation

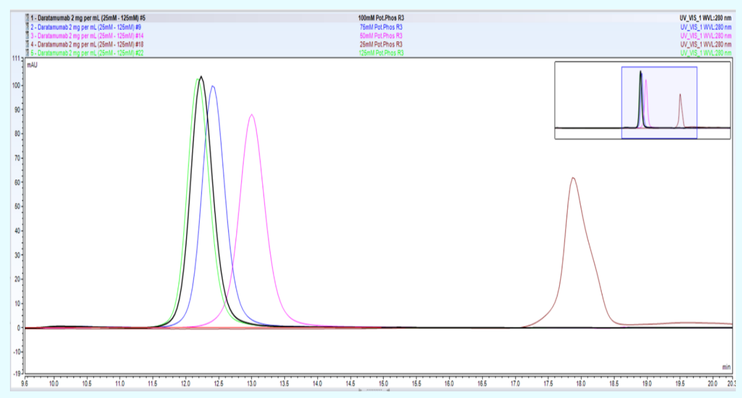


Figure 4: Representative overlay of SE-HPLC chromatogram of Nuzumumab (2 mg/ml) at varying Potassium phosphate buffer concentrations (25 mM – 125 mM).

A sweep from 25 mM to 125 mM Potassium chloride buffer showed vast improvement in all peak characteristics when increasing salt concentration is added to the mobile phase. An optimal concentration of 100 mM Potassium phosphate buffer was used in proceeding experiments with Potassium Chloride.

Potassium Chloride was then added to the mobile phase as a secondary buffer to see if further improvement in peak characteristics could be seen. However, improvements seen by this addition at all concentrations were minimal and could not be seen visually on the chromatogram.

Further interest into the addition of a organic buffer ion the mobile phase will be investigated after my departure from Bath ASU.