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Pharmacology2020:Investigating the effects of the anti-cancer drug Elotuzumab on human multiple myeloma cell lines MM1S and H929 - MARIE BANGURA - University of Westminster

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ABSTRACT
A series of experiments were carried out using Elotuzumab, lenalidomide, and dexamethasone on MM.1S and H929 myeloma cell lines to test their efficacy in vitro and to develop a flow cytometry methodology for the detection of the SLAMF7 receptor on these cells. Elotuzumab is a humanized monoclonal antibody that binds specifically to the SLAMF7 receptor and is normally used in conjunction with lenalidomide and dexamethasone in the treatment of multiple myeloma. Five experiments were run over a period of five weeks. In these experiments, different concentrations of the test drugs singly and in combination were incubated together with cultures of the two cell types. An MTT assay was then used to measure the survival rate of the cells. In addition, the efficacy of old, frozen and fresh batches of Elotuzumab on survival rates was also tested. Finally, the expression of SLAM 7 antibodies was compared between the two cell types using an immunofluorescence and flow cytometric technique. Initial runs failed to produce any significant reduction in survival rates due to Elotuzumab, presumably due to the age of the Elotuzumab sample being used. Use of fresh batches of Elotuzumab yielded the effects associated with the drug which was a reduction in cell viability. Dexamethasone, when used alone or in combinations with the two other drugs produced the greatest reductions in cell survival rates. Lenalidomide only produced moderate reductions in survival rates. There was greater SLAM 7 expression in MM.1S than in the H929 cell types. These results indicate that Elotuzumab alone or in combination with dexamethasone or lenalidomide is only responsible for a small amount of myeloma cell death. Further experiments need to be conducted to determine its efficacy in vivo.

INTRODUCTION
The surface of myeloma cells express a protein called Signalling Lymphocyte-Activating Molecule 7 (SLAMF7). This surface protein is also expressed by natural killer cells. SLAMF7 is a molecule that functions as a receptor. SLAMF7 is considered a biomarker for myelomas because it not expressed on normal tissue cells. Additionally, SLAMF7 is also associated with immune cell inhibition (Guo et al., 2015).

Suppressing SLAMF7 and boosting normal immune cell efficiency is a therapeutic approach that is used for treating multiple myeloma patients. Treatment with the monoclonal anti-SLAMF7 antibody Elotuzumab is one of the most popular methods. The antibodies affect myeloma and natural killer cells through targeting the SLAMF7 receptor protein (Palumbo and Sonneveld, 2015). Thus, the cancerous cells are attacked by antibodies while the normal immune cells are left intact (Veillette and Guo, 2013). Elotuzumab may be used in combination with dexamethasone and lenalidomide. This drug may also be used in the treatment of relapsed multiple myeloma (Tai and Anderson, 2011). Lenalidomide being an immunomodulator is usually administered in combination with the corticosteroid dexamethasone, in order to boost immune cell activity (Weisel, 2016; Einsele and Schreder, 2016).

The number of SLAM7 surface proteins may differ with the type of cell. Thus, the detection of their presence is important in understanding the efficacy of anti-SLAM7 medications. The most commonly used method for their detection is flow cytometry. The most commonly used tests for cell survival is the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; thiazolyl blue) toxicity assay.

The aim of this study is to investigate the efficacy and success of lenalidomide, dexamethasone, Elotuzumab and their combinations on survival of H929 and MM.1S myeloma cells in vitro. This study also investigates the effect of storage time of the drugs on their efficacy. Finally, the study also aims to detect the presence of SLAMF7 antibodies on the surface of H929 and MM.1S cells.

Methodology (Materials and Methods)

Materials

MTT, dexamethasone and lenalidomide were purchased from Sigma -Aldrich company Ltd (New Road, Gillingham, Dorset, U.K.). Signaling lymphocyte activation molecule family 7 (SLAMF7) Mab was purchased from R & D systems (Abingdon, Oxford, U.K.). Monoclonal anti-SLAMF7 antibody, control antibody and anti-Actin antibody, the anti-mouse polyvalent immunoglobulins (GAM)-FITC, antibody produced in goat were purchased from Sigma-Aldrich company Ltd (New Road, Gillingham, Dorset, U.K.). Elotuzumab was a gift from Bristol Squibb company.

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Also, staining solution phosphate buffered saline system (PBS), bovine serum albumin (BSA) were purchased from Sigma-Aldrich Company.

Experiments

A total of 5 experiments to investigate the effect of Elotuzumub, dexamethasone, lenalidomide and their combinations on the survival of the two cell lines were done. Each test solution was added to the wells containing the cell lines and the final volume of medium in each well made up to 200 µL using RPMI 1640 medium. The 96 well plates were then incubated at 37˚c in a humidified incubator for 2 days and the survival rates read.

These were conducted over a 5-week period with one experiment being conducted per week. All experiments were done in triplicate and survival rates recorded. The mean and standard errors of the three survival rates were used in the data analysis. In each experiment a control was added which contained only the cells in their media.

Experiment 1 (Week 1)

In this experiment, Elotuzumab was added to wells containing the two cell lines. The effect of concentration was tested for by adding 25, 50, 100 or 200µg/ml of Elotuzumub and recording the survival rate. The Elotuzumab used in this experiment was an old batch (at least 2 years old).

Experiment 2 (Week 2)

In this experiment, a frozen batch culture of Elotuzumab was employed. The experimental conditions were 25 µg/mL, 50 µg/mL and 100 µg/mL of Elotuzumab, 10mM of dexamethasone and a combination of 10mM dexamethasone with 100 µg/mL Elotuzumab.

Experiment 3 (Week 3)

In this experiment, a fresh batch of Elotuzumab was used. Three concentrations of dexamethasone (0.5mM, 5mM, 50mM and 500mM), one concentration of Elotuzumab (100µg/ml) and a combination of 500mM dexamethasone and 200µg/ml Elotuzumab were tested.

Experiment 4 (Week 4)

In this experiment, one week old (comparatively fresh) Elotuzumab was used. The experimental conditions were Elotuzumab concentrations of 25µg/ml, 50 µg/ml and 100µg/ml, a 10mM concentration of dexamethasone and a combination of 100µg/ml Elotuzumab with 10mM dexamethasone.

Experiment 5 (Week 5)

In this experiment a fresh batch of Elotuzumab was used. The experimental conditions were 25 µg/ml, 50 µg/ml and 100 µg/ml concentrations of Elotuzumab, 10mM of lenalidomide and a combination of 100µg/ml Elotuzumab with 10mM of Lenalidomide.

Determination of cell survival rates

This was done using an MTT assay. The plates were removed from the incubator and most of the supernatant (150µl) was removed carefully from the wells. A volume of 1ml of 5mg/ml MTT was made up in Phosphate Buffered Saline (PBS). Then 2.5 µL MTT reagent solution was added to each well to give a final concentration of 0.25 µg/ml. Mixing was done by pipetting it up and down quickly.

The yellow colouration of the MTT reagent was converted into a blue colour by enzymes of the cells when they were alive after incubation for 4 hours at 37˚c in a humidified incubator. A volume of 25 µl was removed from each well. About 200 µl of a solution containing 80% of DMSO and 20% of ethanol was then added to wells.

The plates were then placed into a thermomax plate reader to determine the intensity of blue colour formation. Absorbance for the reader was set at 570nm.

Indirect Immunostaining of myeloma cells

A total of 5ml of MM.1S and H929 cell lines were pipetted into vacutainer tubes from their culture flasks. These were then spun at 1500rpm for five minutes. The supernatant was poured out and the cells were re-suspended in a 3ml fresh medium.

The cells were counted and adjusted to approximately 500,000/ tube. The cells were then placed into 2 test tubes and 2ml of PBS 0.1%BSA added. One tube contained the MM1S cells while the other contained the H929 cells. The test tubes were then spun at 1500rpm for 5minutes at 4˚c. A volume 150 µL of cold PBS 0.1% BSA was then added to each test tube.

A volume of 10 µL of anti-SLAMF7 antibodies were added into each of the test tubes containing the cells. Two control tubes containing of 10 µL of the antibody (isotype controls) were made. The tubes were incubated on ice for 30 minutes.

After incubation, the cells were washed with 2ml PBS 0.1% BSA and spun at 1500 rpm for five minutes at 4°C. The supernatant was poured out and 150 µL of cold PBS 0.1% BSA added.

A volume of 20ul of GAM-FITC antibody were added into both tubes and mixed by flicking tubes. The tubes were then incubated on ice for 30 minutes.

Finally, 2ml PBS 0.1% BSA were added into each tube and the tubes spun at 1500 rpm for five minutes at 4°C. The supernatant was poured out and 0.5ml PBS 0.1% BSA was added into both tubes.

Flow cytometric analysis

Indirect immunofluorescence flow cytometric analysis was then performed using a Cyan 2 flow cytometer (B&D).

Results

Week 1

The survival rate at 200 µg/ml and 25 µg/ml Elotuzumab was significantly lower than those of the control. All other

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experimental conditions did not differ significantly from the controls.

The 25 µg/ml and 50µg/ml concentrations of Elotuzumab were significantly different with the 50 µg/ml concentration showing higher survival rates than the 25 µg/ml one. In fact, the 50 µg/ml concentration had 100% survival rate which was the same as the control. There was an overlap of the error bars between the 50 µg/ml concentration and the control group. This is shown in the figure below.

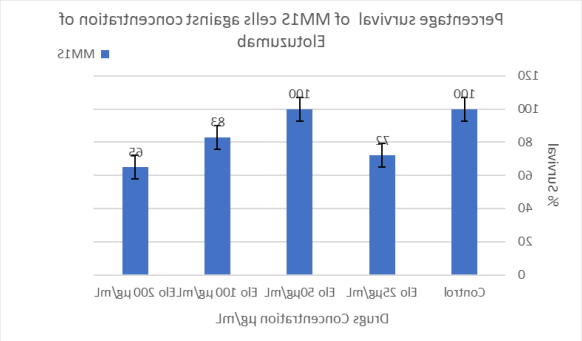

Figure 1A: A bar graph showing the % survival rate of MM.1S cells against the concentrations of Elotuzumab in week 1. Mean and standard error of the mean (SE) for triplicate samples are shown.

The survival rates on the H929 cell lines in this week were all at 100%, except for the 25µg/ml concentration where the concentration was 95%. However, there was no significant difference between this and the rest of the Elotuzumab concentrations or the control.

The survival rates were significantly lower in MM1S cells as compared to H929 cells at all concentrations except the control and 50µg/ml. At this concentration, the survival rates were both at 100%. This is shown in the figure below.

The percentage survival rates of the MM.1S and H929 cells in week 1 are shown in figure 1 B.

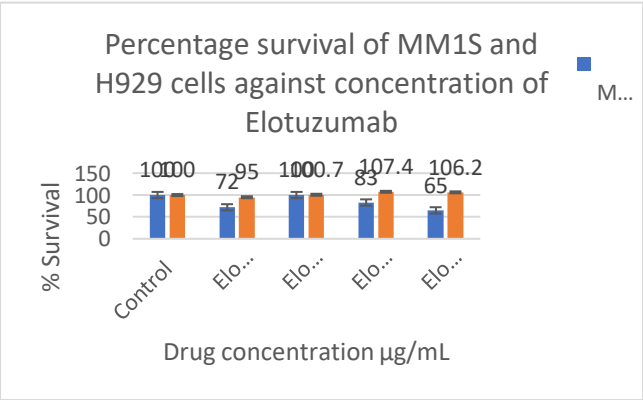

Figure 1B: A bar graph showing the % survival of MM.1S and H929 cells against the concentrations of Elotuzumab in week 1. Mean and standard error of the mean (SE) for triplicate samples are shown.

Week 2

Only at a concentration of 25 µg/ml was there a significantly lower survival rate from the control. There was an overlap of error bars between the survival rates at control, 50 µg/mL and 100 µg/mL Elotuzumab concentration treated cells.

However, the survival rate was not significantly lower at 50 µg/mL as compared to 100µg/mL.

There was a significant difference in the survival rate in MM1S cells treated with Dexamethasone compared to control cells. In addition, the survival rates in cells treated with dexamethasone were significantly lower than the survival rates at all concentrations of Elotuzumab.

When dexamethasone was combined with Elotuzumab the survival rates were also significantly lower than the controls. When compared to survival rates when Elotuzumab was used alone the survival rates in the combination were significantly lower than all concentrations of Elotuzumab.

However, there was no significant difference in the survival rates of MM1S cells treated with dexamethasone alone with those treated with an Elotuzumab/Dexamethasone combination. This is shown in the figure below.

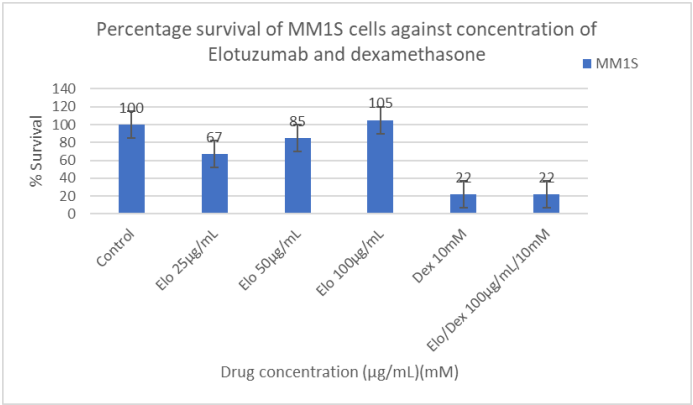

Figure 2A: A Bar graph showing the % survival of MM.1S cells against the concentrations of Elotuzumab and dexamethasone in week 2. Mean and standard error of the mean(SE) for triplicate samples are shown.

The survival rates for H929 cells in experiment 2 were 100% at all concentrations of Elotuzumab when used alone. These survival rates did not differ significantly from each other or from the control.

The survival rate dropped to 81% when a concentration of 10mM dexamethasone was used alone. This was significantly lower than the survival rates when Elotuzumab was used alone. In addition, this was significantly lower than the controls. When a combination of dexamethasone and

Elotuzumab was used the survival, rate was 85%. This was significantly lower than treatments with Elotuzumab alone and the control.

The survival rates when dexamethasone was used alone and when it was used in combination with Elotuzumab were not significantly different.

Survival rates between the two cell types were significantly different at the 25 µg/ml concentration of Elotuzumab. The rates in MM.1S were significantly lower than in H929 cells at this Elotuzumab concentration.

When dexamethasone was used alone the survival rate in MM.1S cells was significantly lower than the survival rate in H929 cells. A similar picture was also seen when dexamethasone was used in combination with Elotuzumab. This is shown in the figure below.

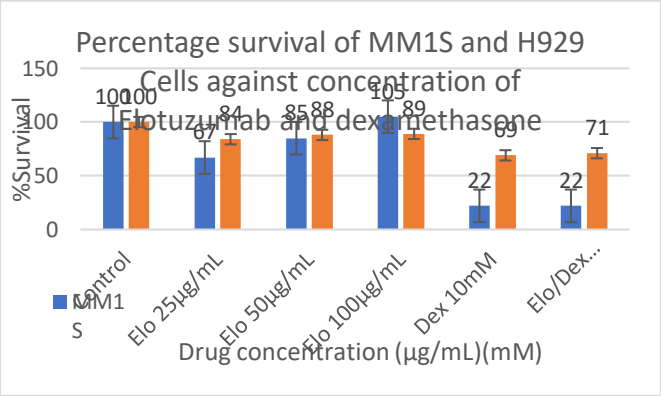

Figure 2B: The Bar graph showing the % survival of MM.1S and H929 cells against the concentrations of Elotuzumab & Dexamethasone in week 2. Mean and standard error of the mean (SE) for triplicate samples are shown.

Week 3

The highest survival rate among the three dexamethasone concentrations was at 0.5mM of dexamethasone (63%) while the lowest was at 500mM dexamethasone (49%).

Survival rates of MM.1S cells at all concentrations of Dexamethasone when used alone did not differ significantly from each other. There was also a trend where the survival rates for the MM.1S reduced as higher concentrations of Dexamethasone were used. It dropped from 63% at 0.5Mm to 49% at 500Mm dexamethasone. All survival rates when dexamethasone was used alone were significantly lower than the control.

When a concentration of 100 µg/ml Elotuzumab was used alone the survival rate was 88%. This did not differ significantly with the control. In addition, it was significantly higher than when dexamethasone was used alone or in combination with it. When a combination of dexamethasone and Elotuzumab was used the survival, rate was 55%. This was significantly lower than the control. It was also significantly lower than when Elotuzumab was used alone. However, this was not significantly different from when dexamethasone was used alone. This is shown in the figure below.

Figure 3A: The Bar graph showing the % survival of MM.1S cells against the concentrations of Elotuzumab & Dexamethasone in week 3. Mean and standard error of the mean (SE) for triplicate samples are shown.

Survival rates for H929 cells were above 80% in all experimental conditions during this week.

Survival rates reduced with increasing concentrations of dexamethasone from 87.1% (0.5Mm) to 80.6% (500mM) when it was used alone. All the survival rates when dexamethasone was used alone were significantly lower than the controls. However, these survival rates were not significantly different from each other.

When Elotuzumab was used alone the survival rate for H929 cells was 98.3%. This did not differ significantly from the control. However, it was significantly higher than all other experimental conditions.

When dexamethasone was used in combination with Elotuzumab the survival rate for H929 cells was 87.5%. This was significantly lower than the control. It was also significantly lower than when Elotuzumab was used alone. However, it was not significantly different from the survival rate when dexamethasone was used alone.

The survival rates for H929 cell lines were significantly higher than those of MM1S in all experimental conditions except for the Elotuzumab 100µg/ml concentration. This is shown in the figure below.

Figure 3B: A bar graph showing the %survival of MM.1S and H929 cells against the concentrations of Elotuzumab and Dexamethasone in the third week. Mean and standard error of the mean (SE) for triplicate samples are shown.

Week 4

Among the Elotuzumab concentrations the lowest survival rate was observed at a concentration of 50µg/ml (52%) while the highest was at 25µg/ml (74%). The survival rate at 50 µg/ml was significantly lower than those at all other concentrations of Elotuzumab. The survival rates when Elotuzumab was used alone were significantly lower than the control.

The survival rate when a combination of Elotuzumab and dexamethasone was used was significantly lower than when 25µg/ml or 100µg/ml of elotuzumab were used alone. However, this survival rate was not significantly different from

the survival rate at the 50 µg/ml concentration of Elotuzumab.

When dexamethasone was used alone the survival rate was 42%. This was significantly lower than the control. It was also significantly lower than the 25 µg/ml and 100 µg/ml Elotuzumab concentrations. However, this survival rate was not significantly different from when a combination of Elotuzumab and dexamethasone was used. This is shown in the figure above.

Among the Elotuzumab concentrations, the lowest survival rate was at 25µg/ml (84%) while the highest was at 100µg/ml (89%). There was no significant difference in the survival rates at all the concentrations of Elotuzumab. However, there was a trend where the survival rates increased as the concentration of Elotuzumab increased. Only the 100µg/ml Elotuzumab concentration was not significantly lower than the control with all other experimental conditions being significantly lower.

When dexamethasone was used alone the survival rate was 69%. It was significantly lower than the control. This survival rate was also significantly lower than when Elotuzumab was used alone.

When a combination of Elotuzumab and Dexamethasone was used the survival, rate was 71%. This was significantly lower than the control, but it did not differ from that when dexamethasone was used alone.

The survival rates of H929 cells were significantly higher than those of MM1S cells in all experimental conditions except the 25µg/ml Elotuzumab concentration. This is shown in the figure below.

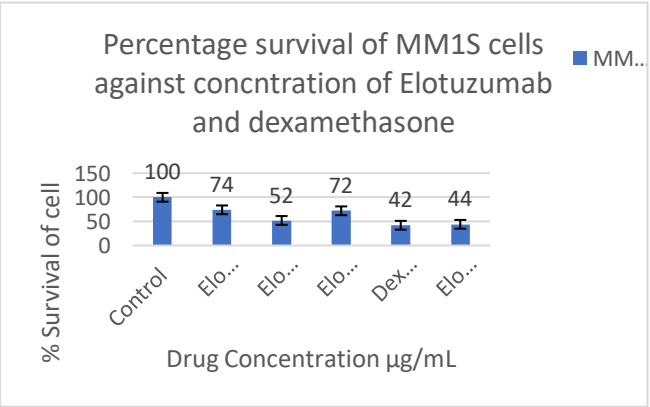

Figure 4B: Bar graph showing the %survival of MM.1S and H929 cells against the concentrations of Elotuzumab and Dexamethasone in the fourth week experiment. Mean and standard error of the mean (SE) for triplicate samples are shown.

Week 5

Among the Elotuzumab concentrations the lowest survival rate was observed at a concentration of 50µg/ml (78%) while the highest was shared by 25µg/ml and 50µg/ml (85%). There was no significant difference among the Elotuzumab concentrations. However, all the survival rates when Elotuzumab was used alone were significantly lower than the controls.

The survival rate when a combination of Elotuzumab and lenalidomide was used was significantly lower than when Elotuzumab were used alone. It was also significantly lower than the control. However, this was not significantly different from that when lenalidomide was used alone. All experimental survival rates were significantly lower than the control. This is shown in the figure below.

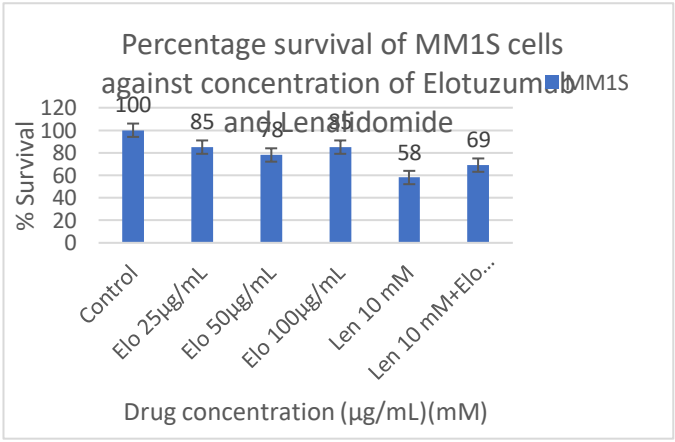

Figure 5A: Bar graph showing the % survival of MM1S cells against the concentrations of Elotuzumab and Lenalidomide fifth week. Mean and standard error of the mean (SE) for triplicate samples are shown.

Among the Elotuzumab concentrations, the lowest was at 25µg/ml (82%) while the highest was at 100µg/ml (87%). There was no significant difference in the rates between all the concentrations of Elotuzumab. The survival rates in all experimental conditions were significantly lower than the control. The survival rate when lenalidomide was used alone or in combination with Elotuzumab did not differ significantly.

The survival rates of H929 cells, were lower than that of MM1S cells at 25µg/ml Elotuzumab concentration. But this was not significant. In all other experimental conditions, it was higher. The only significant difference in survival rates between the two cell types was seen in the 10mM lenalidomide condition and the Elotuzumab/lenalidomide combination. This is shown in the figure below.

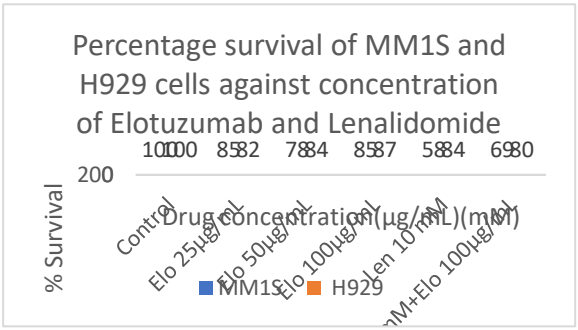

Figure 5B: Bar graph showing the %survival of MM1S and H929 cells against the concentrations of Elotuzumab and Lenalidomide in the fifth week. Mean and standard error of the mean (SE) for triplicate samples are shown.

Pair-wise comparisons

Mean values of the survival rates of MM.1S cells were significantly different between the weekly measurements of the experiment (F=9.032, p<0.001).

The average value of Week 5 was significantly higher than the rest of the four previous weeks (week 5=0.669, week 1-4= 0.375-0.468 range).

Immunofluorescence staining on the flow cytometer

MM1S cells results of immunofluorescence staining on the flow cytometer

The total counts for the number of events detected for MM1S cells were 19176 and 20249 for sample 2 and 1 respectively. Since a total of 500000 cells were in each tube, a total of 3.8% and 4.0% were detected. Dot plots of the data showed two clusters of events. One was located relatively higher than the other. The lower cluster (designated R1 in the diagram below) represented cells that were alive. These were subsequently gated and designated region 1. The region had a total count of 5000 events (26.07% of all events). The mean FITC log (intensity) was 10.53 (sample 2) and 34.44 (sample 1). This is shown in the figures below.

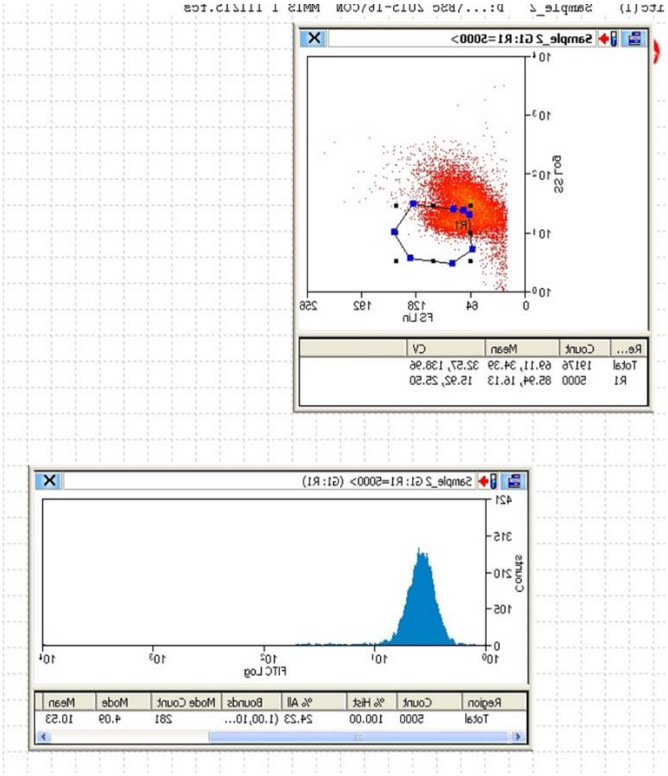

Figure 6A: Flow Cytometry output for MM1S Sample 2.

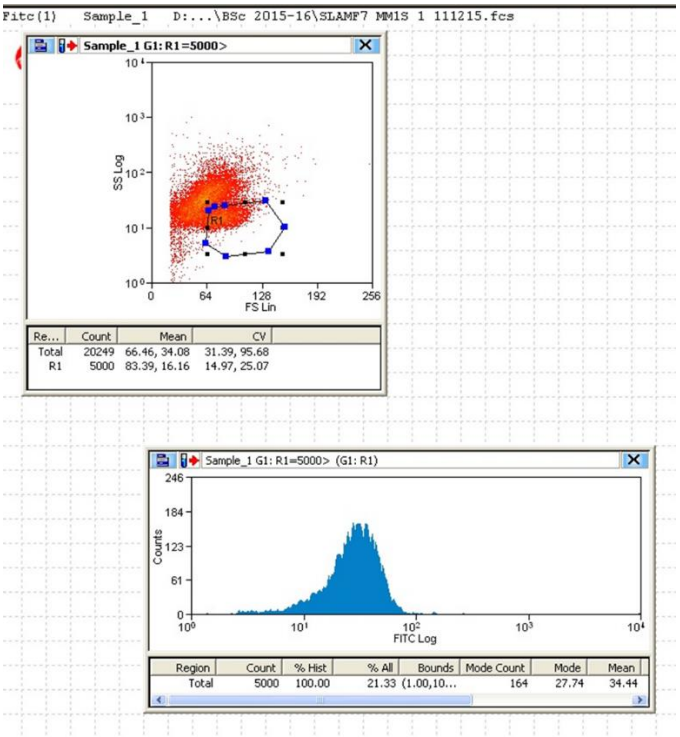

Figure 6 B: Flow cytometry output for MM1S sample 1

H929 cells results of immunofluorescence staining on the flow cytometer

The total counts for the number of events detected for H929 cells were 14185 and 11626 for sample 7 and 8 respectively. Since a total of 500000 cells were in each tube, a total of 2.8% and 2.3% were detected. This was lesser than MM1S cells.

Dot plots from the data showed one cluster of events. The mean FITC log (intensity) was 65.31 (sample 7) and 195.93 (sample 8). This is shown in the figures below.

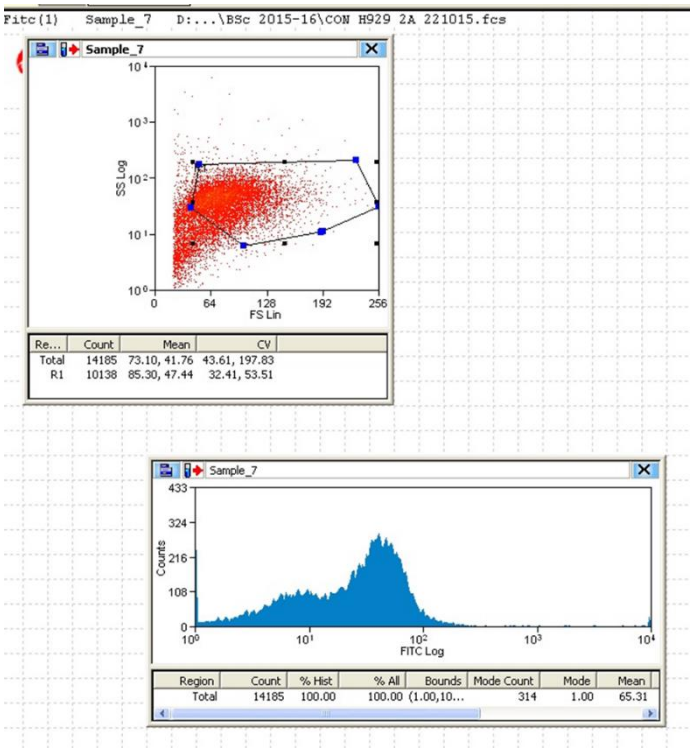

Figure 7 A: Flow cytometry output for H929 sample 7.

For MM1S fluorescence intensity was higher than the H929 because antibody staining is far to the right of the control antibody staining.

Discussion

Multiple Myeloma continues to be a major cause of morbidity and mortality worldwide (Ravindran et al. 2016). Therapy of this type of cancer has included chemotherapy, radiation therapy, surgery or bone marrow transplant. Traditional chemotherapeutic drugs cause side effects. The use of immunomodulation therapies reduces these serious side effects while also helping improve the immune system. Lenalidomide was created as an immunomodulating drug with apoptotic properties. However, it still had some side effects. The discovery of Elotuzumab has promising uses as an alternative. The combination of this drug with other traditional therapies may improve outlooks for multiple myeloma patients.

Elotuzumab is an immune-stimulatory mAb that is directed against the SLAM-7 glycoprotein located on cell surfaces of MM cells. Elotuzumab stimulates MM cell apoptosis by two possible routes of action. It directly triggers the NK cells or induces Antibody-dependent cell-mediated cytotoxicity (ADCC) of the myeloma cells resulting in cell death (Kotla et al., 2009; Lonial et al., 2015; Ma et al., 2007). MM cells are potential drug targets for mAb therapy that promises valuable, significant implications in treating myeloma (Collins et al., 2013; Richardson et al., 2011; Bianchi, Richardson and Anderson, 2015).

Effect of age on Elotuzumab efficacy

In experiment one (on week one) an old batch of the drug was used. In week two a frozen batch of Elotuzumab was used. In week 3 a fresh batch of Elotuzumab was used. However, in this week the focus was on dexamethasone and thus only one concentration of Elotuzumab was used. In week 4 a one week old batch was used while in week 5 a fresh batch was used. The efficacy varied according to how fresh the batch was.

In this experiment, the inhibitory effect of Elotuzumab on cell survival has been very insignificant or nil in the early two trials. Though similar findings were observed for the H929 cells in first two trials, the survival rates for both cell types were significantly different in both trials. This may have been due to the use of old and frozen batches respectively.

In the first two weeks, the efficacy on MM.1S cells was reduced. This was shown by the survival rates of majority of the experimental conditions overlapping with the controls. There was also a higher survival rates among the experimental conditions than in the last two experiments. The survival rates for H929 cells in these first two weeks remained at almost 100%.

Efficacy for MM.1S in the last two weeks was greatly increased. Survival rates were significantly lower than the controls and there was a dose related response to the therapy. This was seen best for H929 cells where the survival rates were significantly lower than the controls.

While a lot of studies have dealt with efficacy in vivo (Gavriatopoulou et al., 2017; Wang et al., 2016), none has considered the age of batch or other storage conditions. This is important especially for biological preparations (European Medicines Agency 2013).

The mechanism of action of Elotuzumab is NK cell dependent, thus first it induced NK cells via ADCC and bound itself to SLAMF7 receptor expressed on NK cells (Guo et al., 2015).

Dexamethasone

Dexamethasone is a corticosteroid medication that is used widely in medicine. In cancer cells, it acts either alone or synergistically with other drugs to cause cell death. The mechanism of cell death has been proposed as autophagy (Laane et al., 2009). Dexamethasone can also cause cell death using other mechanisms (Lanshakov et al,. 2016). In all experiments where dexamethasone has been used it has caused the most profound reduction in survival rate. This has been true both when used alone or in combination with Elotuzumab or Lenalidomide. And it has proved efficacious both on MM.1S cells and H929 cells.

In week 3 where dexamethasone was the focus of the experiment, there was a clear dose response. This was seen with reducing survival rates as the concentration was increased. This was seen for both MM.1S cells and in H929 cells. All survival rates were significantly lower than the controls.

Survival rates when dexamethasone was used (weeks 2-4) were significantly lower than the controls. This was true for both cell types as well as when used in combination with other drugs. However, the effect was much more pronounced in MM1S cells than in H929 cells. Dexamethasone produced the most significant reduction in survival rates of all test drugs.

It has been observed that Elotuzumab is efficient in eliminating the MM cells in vivo and along with Lenalidomide/Dexamethasone its efficacy gets improved. The combination of Dexamethasone and Lenalidomide contributes in hindering of cell proliferation as well as stimulation of apoptosis of MM cell lines (MM1S and H929 cell lines) (Klippel et al., 2007).

In the second week trial, the cell survival rate when Dexamethasone was used alone or combined with Elotuzumab concentration declined significantly showing the improved efficacy. Similar results have been found in other in vitro studies (Guo et al. 2015). Even when fresh batches of Elotuzumab were used, the survival rates never went lower than those when dexamethasone was used. This has been hypothesized to be because another cofactor (may be found in vivo) is needed for Elotuzumab to work. Dexamethasone might not require any other factor for its efficacy. The failed effect of the Elotuzumab may also have been due to the use of 2 years old or frozen culture batches.

The other results showed that MM1S and H929 cells were stimulated by Elotuzumab and anti-SLAMF7 because Elotuzumab induces proliferation too. In the other trials, it is seen the comparatively lower dose of Dexamethasone could reduce the viability cells. The effects were more effective when fresh cultures were used.

MM1S cells were more sensitive in comparison to H929 cells, and one cell line showed substantial response against one or mixture of both drugs (Drach et al., 2007). The major finding was that combination of Elotuzumab showed more effects when used in combination with Dexamethasone and Lenalidomide. In this experiment, a dose-dependent pattern of effect was observed that is a feature of immune cell activation (Hipp et al., 2016).

Lenalidomide

This is a thalidomide derivative that has been in use in cancer treatment for a long period. This drug works by an immunomodulatory effect as well as stopping blood vessel formation in growing cancer tissues (anti-angiogenic). It also directly causes cell death by apoptosis (Tageja 2011). The drug was the focus of experiment 5. Lenalidomide when used alone caused a significant reduction in survival rates than when Elotuzumab was used alone. This drug has been used in combination with Elotuzumab and dexamethasone for the treatment of multiple myeloma (Richardson et al. 2015). When this drug was used in combination with elotuzumab there was still significant reduction in survival rate. However, this reduction was not significantly different from the survival rate when Lenalidomide was used alone.

Pairwise Comparisons

It was also observed that pair-wise differences are significant in all the weeks, except week 2 and 4. Thus significant differences were seen between week 1 and week two, week 2 and week 3, week 1 and week 3, week 3 and week 4, week 3 and week 5, week 4 and week 5, week 1 and week 4 and week 1 and week 5.

Mean values of the survival rates of MM.1S cells were significantly different between the weekly measurements of the experiment. The average value of Week 5 was significantly higher than rest of the four previous weeks. In week 5 a fresh batch of Elotuzumab was used. In addition, Lenalidomide was used. This may suggest that using a fresh batch of Elotuzumab and the combination with lenalidomide might have resulted in statistically lower survival rates in these cells.

Flow cytometry

Flow cytometry was conducted to investigate the expression of signaling lymphocytic activation molecule F7 (SLAMF7). This protein is also called CD319. The protein is important in regulating the adhesion of both T and B lymphocytes (Punnonen et al., 1997; Cocks et al., 1995). It is also implicated in activation of Natural Killer cells (Guo et al,. 2015). Genes encoding this protein are found to be highest in the spleen, bone marrow, lymph nodes and the tonsils (Uhlén et al., 2015). In multiple myeloma cells it is highly expressed (Guo et al., 2015).

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The flow cytometry results indicated that SLAM 7 expression was greater for MM.1S cells than for H929 cells. This was seen as increased intensity in immunofluorescence from FITC labelled antibodies to the protein. No study has compared the SLAM 7 expression between these two cell types directly. This may have been a contributing factor in the reduced effectiveness of Elotuzumab in this study. Studies done using another MM cell line U266 showed good response (as demonstrated by reduced survival rates) to the drug (Guo et al., 2015).

In conclusion, the efficacy of Elotuzumab is greatly reduced both in MM.1S and H929 myeloma cell lines. This may be due to storage conditions or more likely requiring an in vivo Co factor to work. Dexamethasone showed the greatest efficacy while lenalidomide showed moderate efficacy in vitro. Also MM.1S cells have higher levels of SLAM 7 molecules than H929.

Further, in vivo studies are needed to create a better picture on the efficacy of these drugs as a prerequisite to their use in therapy.

References

Aldrich fine chemicals I sigmaAldrich.com. Available from http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=CDS022536&brand. [Accessed 6 March 2017].

ATCC: The Global Bio resource Centre. Available from https://www.lgcstandardsatcc.org/Search_Results.aspx?dsNav=Ntk:PrimarySearch%7 cMM1S%7c3%7c, Ny: True,Ro:0,N:1000552&searchTerms=MM1S&redir=1[Accessed 6 March 2017].

Bianchi, G., Richardson, P.G. and Anderson, K.C., (2015). Promising therapies in multiple myeloma. The American society of Hematology, 126(3), 300-310

Cocks, B.G., Chang, C. C., Carballido, J. M., Yssel, H., de Vries, J. E. and Aversa, G. (1995). A novel receptor involved in T-cell activation. Nature, 376 (6537), 260–263.

Collins, S.M., Bakan, C.E., Swartzel, G.D., Hofmeister, C.C., Efebera, Y.A., Kwon, H., Starling, G.C., Ciarlariello, D., Bhaskar, S., Briercheck, E.L. and Hughes, T., (2013). Elotuzumab directly enhances NK cell cytotoxicity against myeloma via CS1 ligation: evidence for augmented NK cell function complementing ADCC. Cancer Immunology, Immunotherapy, 62 (12), 1841-1849.

Drach, J., Kaufmann, H., Ackermann, J., Heller, G., Seidl, S. and Zoechbauer-Mueller, S., (2007). Chromosomal and epigenetic abnormalities in MGUS and MM post-MGUS. In Haematologica-The Haematology Journal, 92 (6) 8-9.

Einsele, H. and Schreder, M. (2016). Treatment of multiple myeloma with the immunostimulatory SLAMF7 antibody elotuzumab. Therapeutic Advances in Hematology, 7(5), 288-301.

European Medicines Agency, (2013). Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance : non-clinical and clinical issues,

Gavriatopoulou, M., Terpos, E., Kastritis, E. and Dimopoulos, M. A. (2017). Efficacy and safety of elotuzumab for the treatment of multiple myeloma. Expert Opinion on Drug Safety, 16 (2), 237–245. Available from: http://dx.doi.org/10.1080/14740338.2017.1279603.

Guo, H., Cruz-Munoz, M.E., Wu, N., Robbins, M. and Veillette, A. (2015). Immune Cell Inhibition by SLAMF7 Is Mediated by a Mechanism Requiring Src Kinases, CD45, and SHIP-1 That Is Defective in Multiple Myeloma Cells. Molecular and Cellular Biology, 35(1), 41–51. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4295394/.

Hipp, S., Tai, Y.T., Blanset, D., Deegen, P., Wahl, J., Thomas, O., Rattel, B., Adam, P.J.,Anderson, K. and Friedrich, M. (2016). A novel BCMA/CD3 bi-specific T cell engager for the treatment of multiple myeloma induces selective lysis in vitro and in vivo. Leukaemia.

Klippel, S., Jakubikova, J., Jenkins, L., Negri, J.M., Tesmenitsky, Y., Rooney, M., Delmore, J., McMillin, D.W., Selliah, R., Robbins, P. and Mitsiades, N. (2007). In Vitro and In Vivo Anti-Myeloma Activity of PRLX, an Orally-Bioavailable Agent against Cells with Constitutive Activation of Ras or Its Downstream Pathway. Blood, 110 (11), 540- 540.

Kotla, V., Goel, S., Nischal, S., Heuck, C., Vivek, K., Das, B. and Verma, A. (2009). Mechanism of action of lenalidomide in hematological malignancies. Journal of hematology and oncology, 2 (1), 36.

Laane, E., Tamm, K.P., Buentke, E., Ito, K., Kharaziha, P., Oscarsson, J., Corcoran, M., Björklund, A.C., Hultenby, K., Lundin, J., Heyman, M., Söderhäll, S., Mazur, J., Porwit, A., Pandolfi, P.P., Zhivotovsky, B., Panaretakis, T. and Grandér, D. (2009). Cell death induced by dexamethasone in lymphoid leukemia is mediated through initiation of autophagy. Cell Death Differ, 16(7), 1018–1029. Available from: http://dx.doi.org/10.1038/cdd.2009.46.

Lanshakov, D.A., Sukhareva, E., Kalinina, T. S. and Dygalo, N. (2016). Dexamethasone-induced acute excitotoxic cell death in the developing brain. Neurobiology of disease, 91, 1–9.

Lonial, S., Dimopoulos, M., Palumbo, A., White, D., Grosicki, S., Spicka, I., Walter- Croneck, A., Moreau, P., Mateos, M.V., Magen, H. and Belch, A. (2015). Elotuzumab therapy for relapsed or refractory multiple myeloma. New England Journal of Medicine, 373(7), 621-631.

This Work is Partly Presented at 33 rd World Congress Scheduled During August 26-27,2020 at Barcelona,Spain

Ma, H., Ziegler, J., Feng, R.T., Lentzsch, S. and Mapara, M. (2007). Cucurbitacin I (JSI-124) Has Potent Anti-Myeloma Effects Independent of Its Inhibition of JAK2-Induced STAT3 Activation. Blood, 110 (11), 2521-2521.

Novel pH, A.I.M.U., (2009). Article of Significant Interest Selected from This Issue by the Editors. Eukaryotic Cell, 8(3), 261.

Palumbo, A. and Sonneveld, P. (2015). Preclinical and clinical evaluation of elotuzumab, aSLAMF7-targeted humanized monoclonal antibody in development for multiple myeloma. Expert review of hematology, 8 (4), 481-491.

Punnonen, J., Cocks, B.G., Carballido, J.M., Bennett, B., Peterson, D., Aversa, G. and de Vries, J.E. (1997). Soluble and membrane-bound forms of signalling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. The Journal of experimental medicine, 185 (6), 993–1004.

Ravindran, A., Bartley, A.C., Holton, S.J., Gonsalves, W.I., Kapoor, P., Siddiqui, M.A., Hashmi, S.K., Marshall, A.L., Ashrani, A.A., Dispenzieri, A., Kyle, R.A., Rajkumar, S.V. and Go, R.S. (2016). Prevalence, incidence and survival of smoldering multiple myeloma in the United States. Blood Cancer Journal, 6 (10), e486. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5098258/.

Richardson, P.G., Jagannath, S., Moreau, P., Jakubowiak, A.J., Raab, M.S., Facon, T., Vij, R., White, D., Reece, D.E., Benboubker, L., Zonder, J., Tsao, L.C., Anderson, K.C., Bleickardt, E., Singhal, A.K. and Lonial, S. (2015). Elotuzumab in combination with lenalidomide and dexamethasone in patients with relapsed multiple myeloma: final phase 2 results from the randomised, open-label, phase 1b-2 dose-escalation study. Lancet Haematol, 2. Available from: http://dx.doi.org/10.1016/S2352-3026 (15)00197-0.

Richardson, P.G., Lonial, S., Jakubowiak, A.J., Harousseau, J.L. and Anderson, K.C. (2011). Monoclonal antibodies in the treatment of multiple myeloma. British journal of haematology, 154 (6), 745-754.

Tageja, N., 2011. Lenalidomide - current understanding of mechanistic properties. Anti-cancer agents in medicinal chemistry, 11 (3), 315–326.

Tai, Y.-T. and Anderson, K.C. (2011). Antibody-based therapies in multiple myeloma. Bone marrow research, 2011.

Tai, Y.-T., Dillon, M., Song, W., Leiba, M., Li, X.-F., Burger, P., Lee, A.I., Podar, K.,Hideshima, T., Rice, A.G., Abbema, A.V., Jesaitis, L., Caras, I., Law, D., Weller, E., Xie, W., Richardson, P., Munshi, N.C., Mathiot, C., Avet-Loiseau, H., Afar, D.E.H. and Anderson, K. C. (2008). Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. The American Society of Hematology, 112 (4), 1329-1337.

Uhlén, M. et al., 2015. Tissue-based map of the human proteome. Science, 347(6220), 1260419. Available from: http://science.sciencemag.org/content/347/6220/1260419.abstract.

Veillette, A. and Guo, H. (2013). CS1, a SLAM family receptor involved in immune regulation, is a therapeutic target in multiple myeloma. Critical reviews in oncology/hematology, 88 (1), 168-177.

Wang, Y., Sanchez, L., Siegel, D. S. and Wang, M. L. (2016). Elotuzumab for the treatment of multiple myeloma. Journal of Hematology & Oncology, 9(1), 55. Available from: http://dx.doi.org/10.1186/s13045-016-0284-z.

Weisel, K. (2016). Spotlight on elotuzumab in the treatment of multiple myeloma: the evidence to date. OncoTargets and therapy, 9, 6037.