



# INVESTIGATING THE METABOLOMICS OF TUMORICIDAL PROTEIN-FATTY ACID COMPLEXES ON BONE CANCER CELLS

## AUTHORS

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## INTRODUCTION

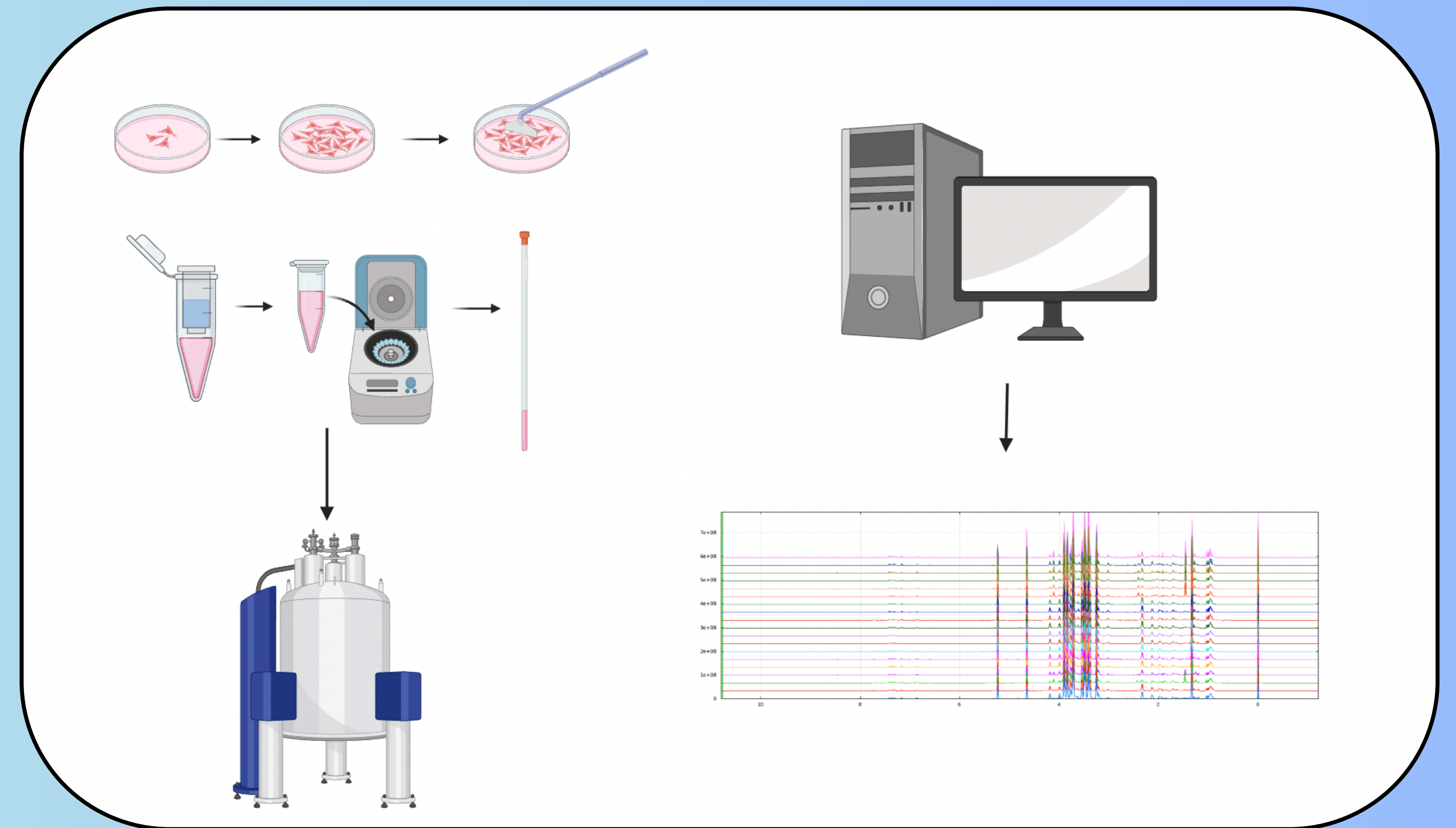
Recently, a highly selective protein-based chemotherapeutic agent HAMLET/BAMLET (Human/Bovine Alpha-lactalbumin Made Lethal to Tumor cells) has shown selective cytotoxicity towards only cancer cells, leaving normal, undifferentiated cells unharmed. Newly, it was shown to be efficacious against bladder cancer, passing phase I/II clinical trials. [1] [2]. It is a protein-fatty acid complex, made up of  $\alpha$ -lactalbumin and oleic acid, which appears to act much faster (60-90 min after administration) than conventional small-molecule therapeutics. The only way that such a drug can rapidly influence cells is through its metabolism. [3] Osteosarcoma (OS) is a form of bone cancer that primarily occurs in long bones, such as tibia or femur, and is seen in approximately 2.4% of paediatric cancers. [4]

## OBJECTIVE

- The objective of this research project is to utilise NMR metabolomics to monitor the changes in the cellular metabolites in the different sample groups. Metabolomics is the study of profiling molecules, known as metabolites, within cells of a biological system. [5] NMR is used to identify and accurately quantify hundreds/thousands of metabolites found in analytes (sera, urine, cellular lysates, and culture media).
- The aim of this experiment is to determine the metabolic impact that BAMLET has on the cells of human osteosarcoma (U2OS cell line), and how this information can be used to produce personalised medicine for the treatment of cancer.

## METHODOLOGY

- U2OS cell line was cultured and separated into BAMLET-treated and water controls, each being exposed to BAMLET or water for certain time lengths.
- Samples were prepared into NMR tubes and  $^1\text{H}$  NMR spectra were acquired using an Agilent Technologies Ultra High Field 18.8 T 800 MHz NMR spectrometer.
- The spectra were preprocessed using NMRProcFlow 1.4.20. Data filtering, normalisation, and scaling were applied using MetaboAnalyst 5.0.
- Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), one-way ANOVA analysis and volcano plots were generated using MetaboAnalyst 5.0. These were used to compare the metabolic profiles between the sample groups. Both multivariate analysis between all the groups and pairwise analysis between the 2hr sets and 24h sets were carried out.



## RESULTS

### Multivariate analysis

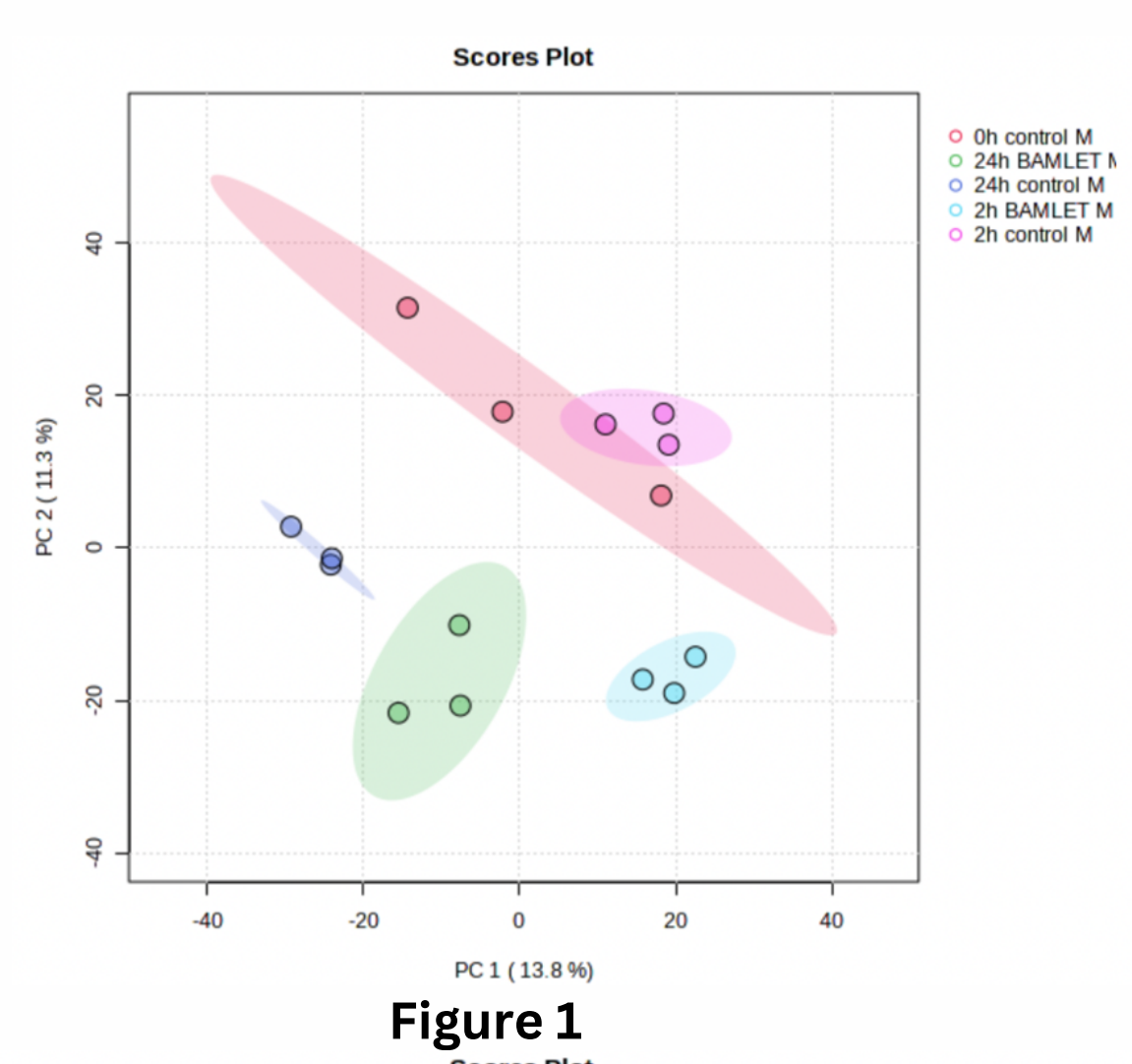


Figure 1 shows the PCA Scores Plot from multivariate comparison of all the sample groups. Clear separation is seen between the groups. The metabolic profile of each group is illustrated by their 95% confidence ellipsoids showing they are all exhibiting distinct metabolic profiles.

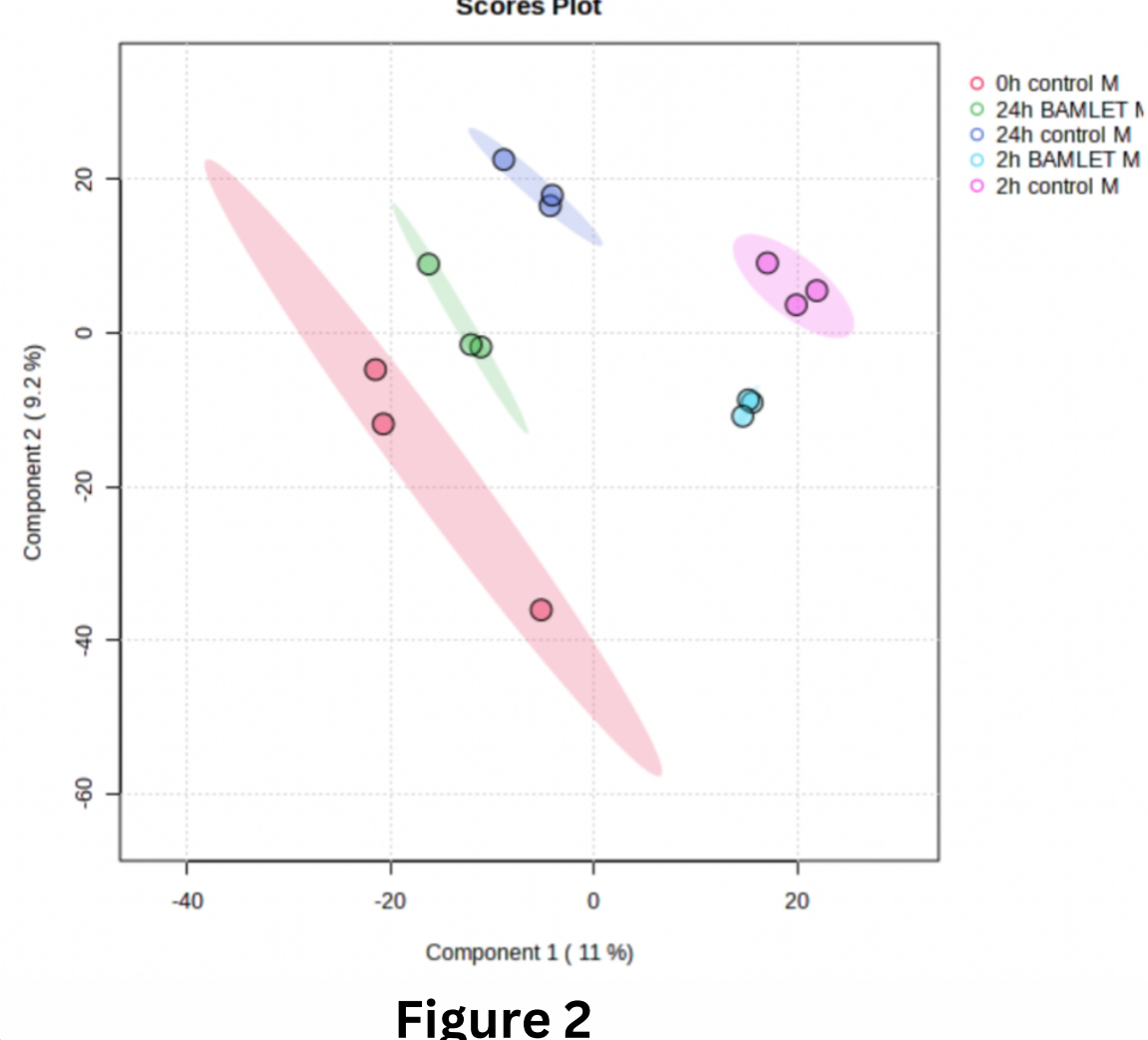


Figure 2 shows the PLS-DA Scores plot from multivariate comparison between the sample groups. Clear separation is seen between the samples. The predictive value calculated from the analysis ( $Q^2 = 0.04$ ) was low and it was found that this model is not predictive.

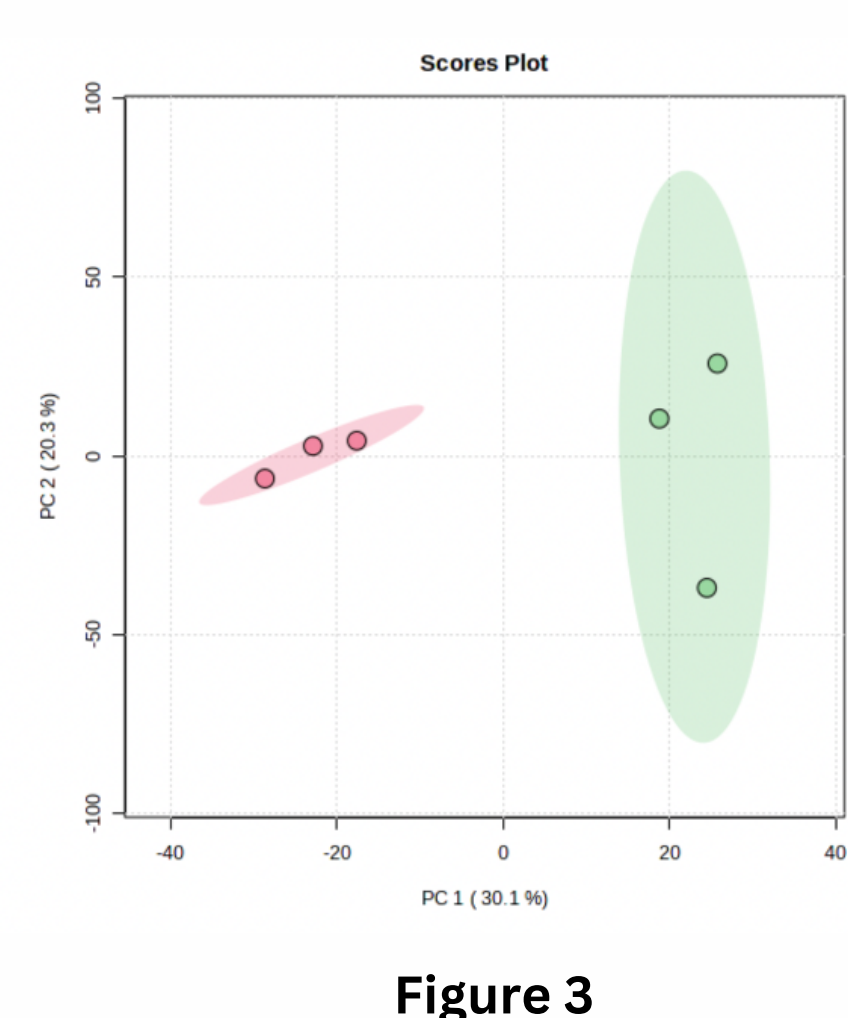


Figure 3

Figure 3 shows the pairwise analysis between the 2h BAMLET vs. 2h control samples. The PCA Scores plot displays a clear separation between the two groups. The metabolic profile of each group is illustrated by the 95% confidence ellipsoids. The results of this analysis show that these two groups present distinct metabolic profiles.

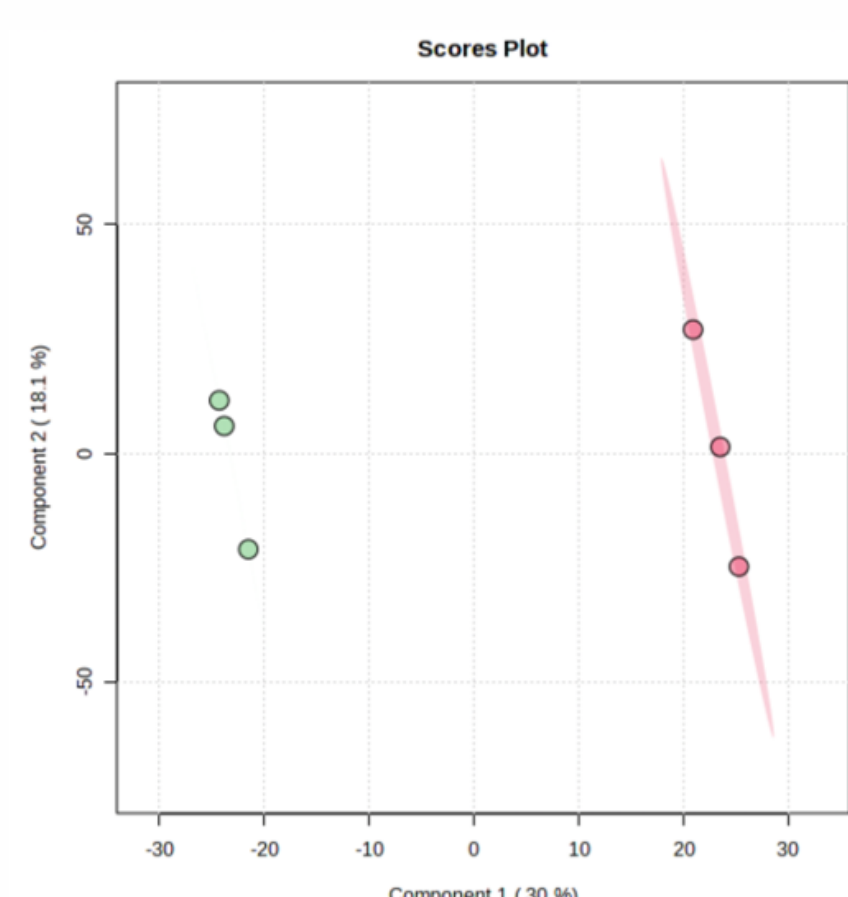


Figure 4

Figure 4 shows the PLS-DA Scores plot generated from pairwise analysis between 2h BAMLET vs. 2h control samples. There is clear separation seen between the two sample groups in question. The predictive value calculated during the analysis ( $Q^2 = 0.41$ ) indicated that this is a predictive model.

### Pairwise analysis

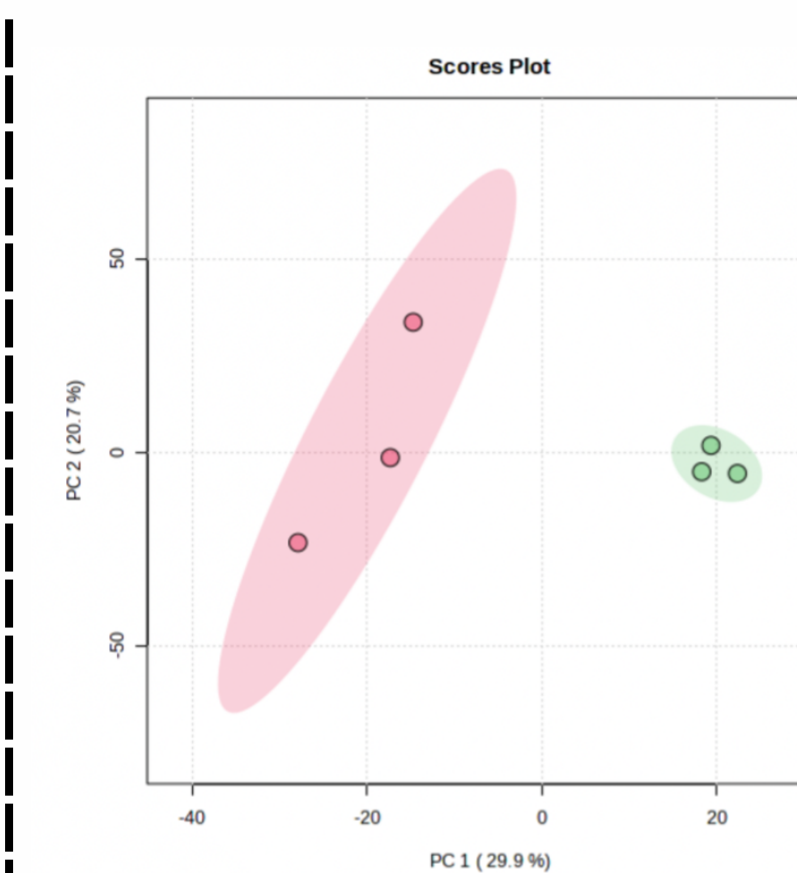


Figure 5

Figure 5 shows the PCA Scores plot from the pairwise analysis between the 24h BAMLET vs. 24h control samples. The metabolic profile of each group is clearly distinct as seen from the 95% confidence ellipsoids illustrated.

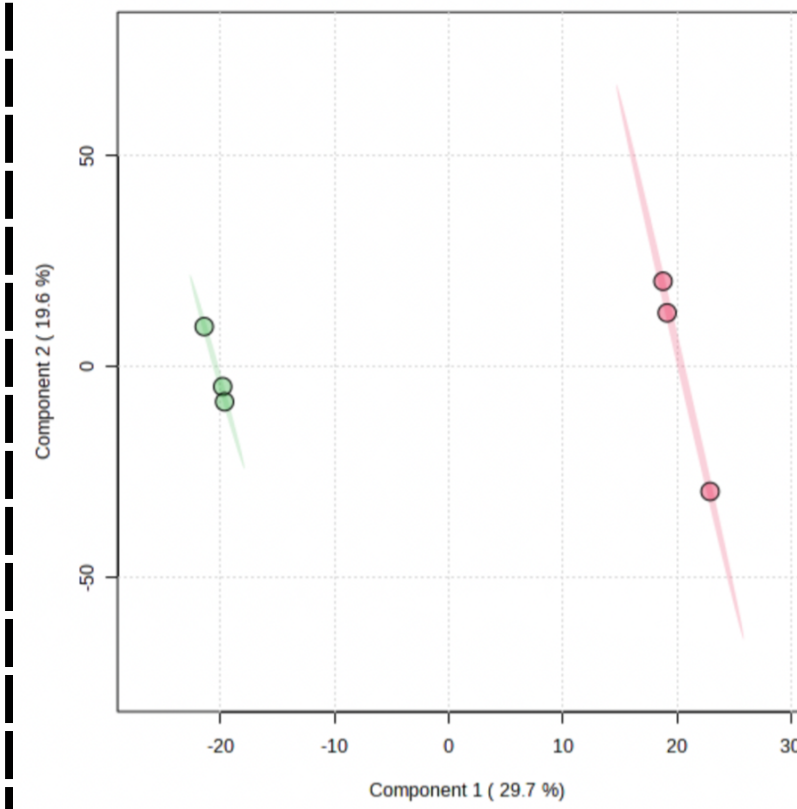


Figure 6

Figure 6 shows the PLS-DA scores plot generated from the pairwise analysis of 24h BAMLET vs. 24h control samples. The separation between the two groups is clearly illustrated in the plot. The predictive value of the model ( $Q^2 = 0.38$ ) was calculated during the PLS-DA analysis and the model was found to be predictive.

## CONCLUSION

- The results show clear metabolic profile differences between the BAMLET-treated groups and control groups, further highlighting that BAMLET does influence the metabolism of the osteosarcoma cells.
- The next step in this research is to find out which metabolites were significantly different between the groups, and how this information could be used for the production of a personalised therapeutic for human osteosarcoma.

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## RELATED LITERATURE

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