Modulation of Tumour Microenvironment and Metastatic Potential by Pyrvinium Pamoate in Triple Negative Breast Cancer Mouse Model

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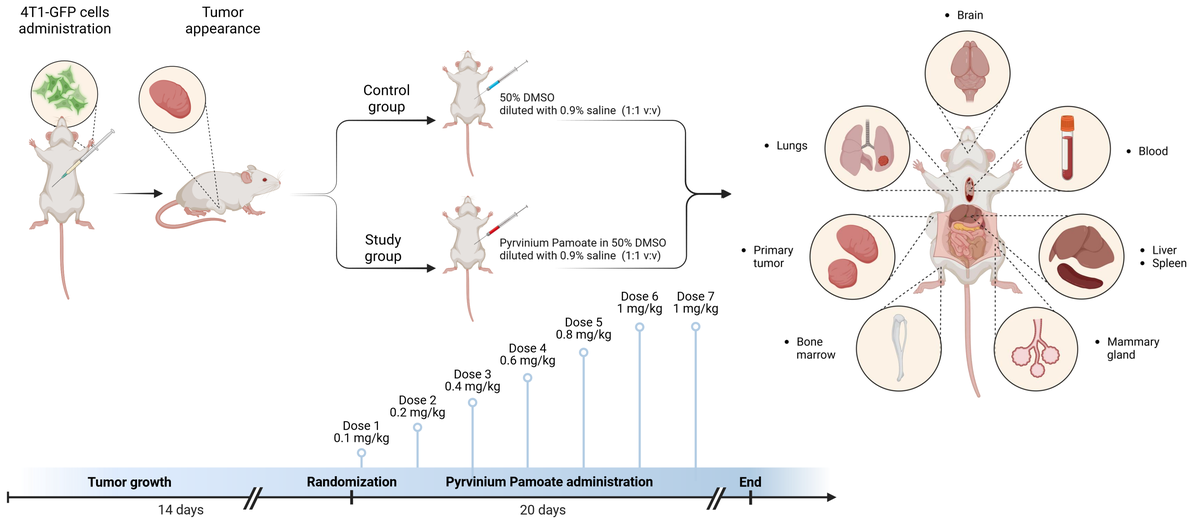
Pyrvinium Pamoate (PP) is an FDA-approved anthelminthic drug currently investigated for its anticancer potential, including in breast cancer [1]. **Triple Negative Breast Cancer (TNBC)** is the most aggressive breast cancer subtype, with limited treatment options. One of the key mechanisms driving its progression is **epithelial-mesenchymal transition (EMT)**, associated with metastasis, treatment resistance, and metabolic reprogramming [2].

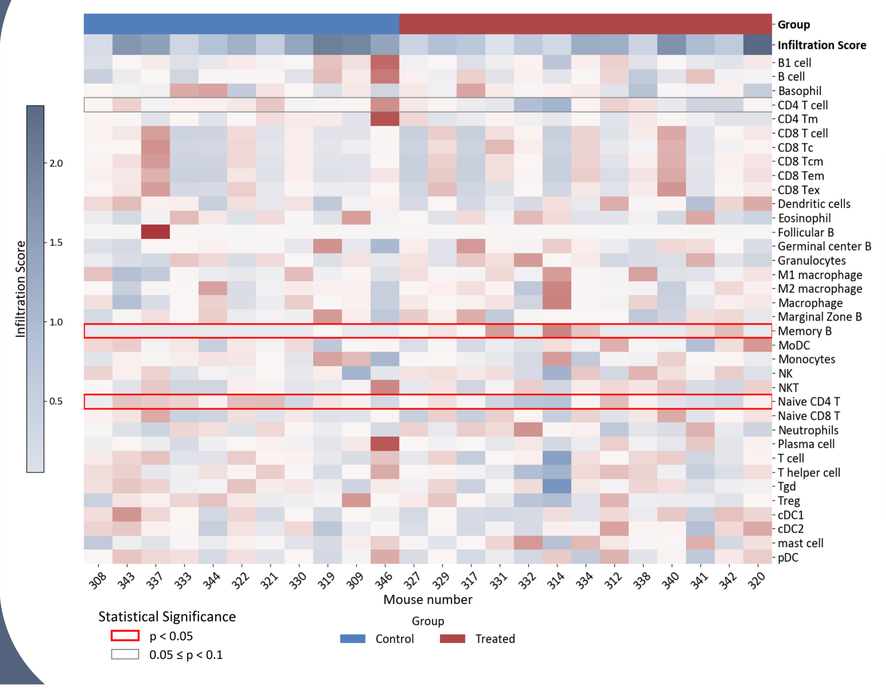
Mouse model of Triple Negative Breast Cancer

orthotopic administration of 4T1-GFP/luc to Balb/c mice



Female BALB/c mice (6–8 weeks old, n = 40) were orthotopically injected with 25,000 4T1-GFP cells. Mice were divided into Treated (n = 20; PP 0.1–1 mg/kg, i.p.) and Control (n = 19; DMSO/PBS) groups, with administrations every other day. Blood was collected under general anaesthesia, tumours and organs were collected post-mortem.

Infiltration score analysis in primary tumours



Z-scored immune cell abundance in primary tumours. Mice in both treated and control groups showed individual differences in immune cell proportions in primary tumours. PP-treated tumours showed increased memory B cells and reduced naive CD4+ and CD4+ T cells compared to control. Infiltration Score calculated using ImmuCellAI[3]. Statistical significance determined by Mann-Whitney U test.

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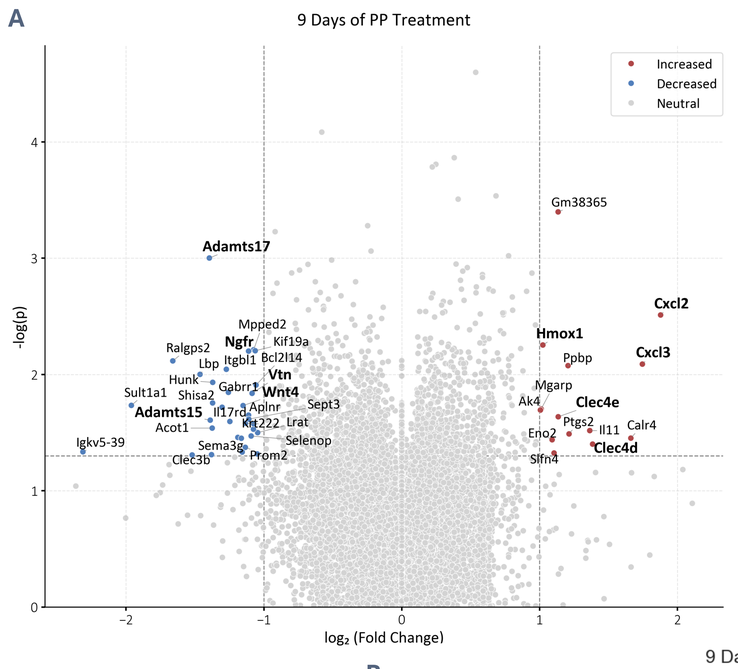
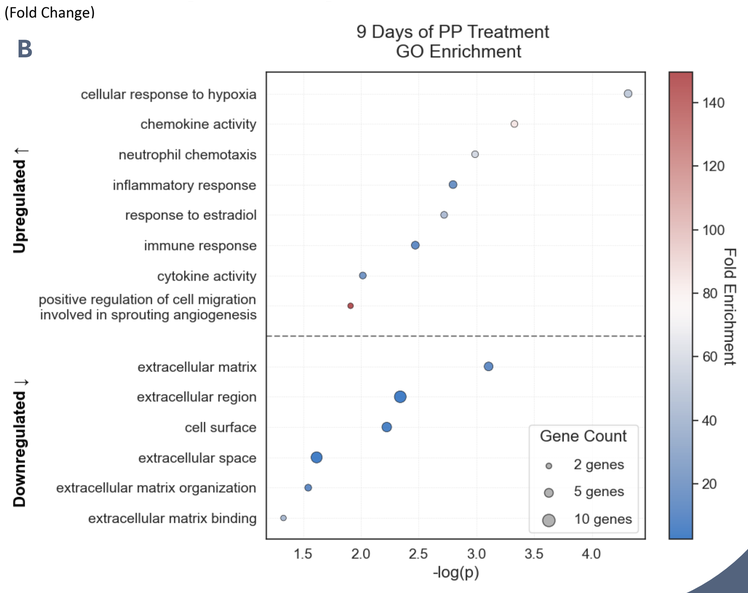
RNA-seq analysis from primary tumour

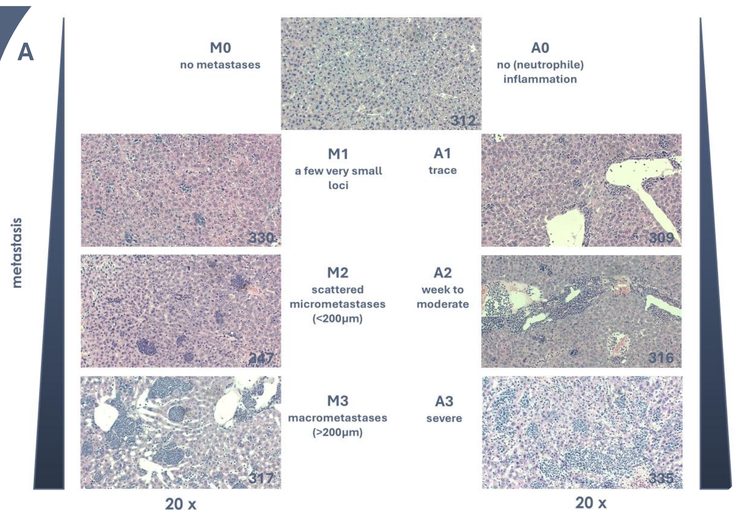


A. Differential gene expression in frozen primary tumours from mice treated with Pyrvinium Pamoate (≥ 9 days), compared to control mice. RNA-seq revealed 32 downregulated and 14 upregulated genes (Welch's t-test, adjusted p < 0.05).

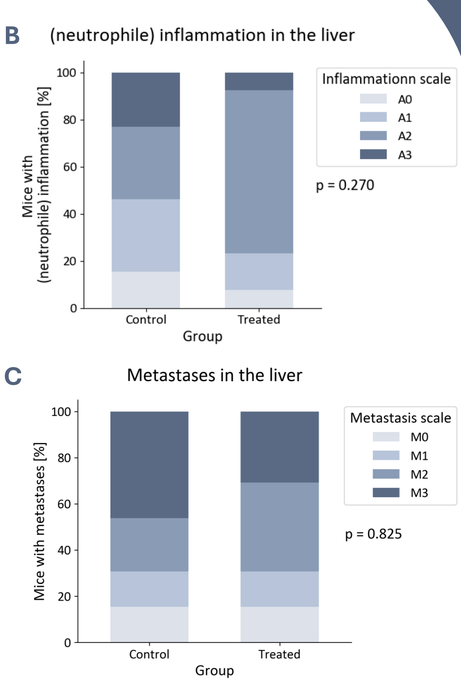


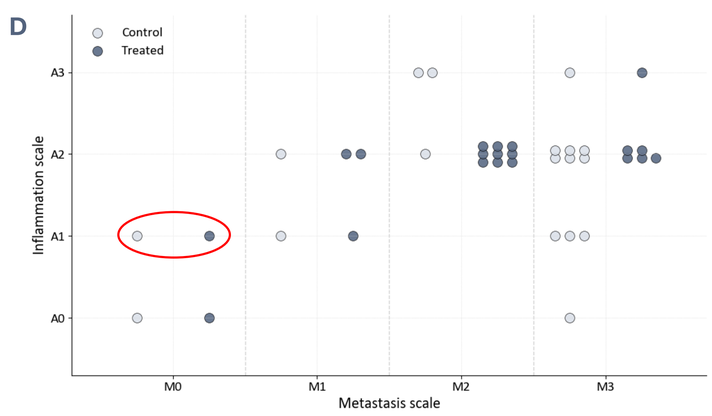
B. The gene ontology for the same group of samples suggests that treatment with PP enhances the immune response and stress-related processes and inhibits extracellular matrix organisation and associated components.

Histopathological assesment of livers



A. Metastasis and inflammation in mice livers. Livers were fixed in FFPE and stained with H&E. Left – metastasis scale, right – (neutrophilic) inflammation scale.

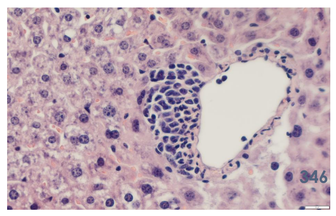




D. Comparison of the level of metastasis with the level of neutrophilic inflammation in the liver in treated and control mice. Inflammatory foci composed of neutrophils were often present in tumour cell–free hepatic areas (**E**).

B. Distribution of neutrophilic inflammation in the liver in treated and control mice (≥9 days treatment).
C. Distribution of metastasis in the liver in treated and control mice (≥9 days treatment).
Statistical significance determined by Pearson's Chi-square test.

E



Conclusions and further perspectives:

- PP altered gene expression in primary tumors by **enhancing immune response** and **reducing extracellular matrix pathways**.
- PP-treated tumours showed **increased memory B cells** and **reduced naive CD4+ and CD4+ T cells** compared to control.
- Liver histology revealed a shift in neutrophil inflammation, with some liver areas showing **inflammation without metastases**.
- Further analysis of neutrophil polarization in primary tumours and livers is planned to clarify their role in tumour progression and treatment response.

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References:

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