

Tracking Neutrophils in the Early Metastatic Niches Using Ex Vivo Live Imaging

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Project location: Cancer Research UK Scotland Institute

Lay Summary:

Metastasis is the leading cause of cancer mortality. Neutrophils, a type of immune cell, play important roles in cancer with both potent anti- and pro-tumour activities. They are produced in both the bone marrow and the spleen, and recent research has established that their phenotype was much more heterogenous than we previously thought. Our lab has also shown that the spleen may serve as an important extramedullary production site in cancer, capable of producing neutrophils distinct from those that originated in the bone marrow. My summer project explored this by studying how neutrophils from the bone marrow and spleen behave and interact with tumour cells in the lung at early stages of metastasis. Using confocal microscopy ex vivo live imaging, I tracked neutrophil movements in lung tissue from a mouse model of metastasizing pancreatic cancer. I found that neutrophils from both sources clustered near tumour cells, but spleen-derived neutrophils may have higher arrest times and moved slower when near tumour cells, suggesting a potential role in early metastasis.

Aim of project

To investigate whether bone marrow- and spleen-derived neutrophils showed distinct motility behaviours in the lung early-metastatic niche, and how these differences influence tumour cell interactions.

How the aim was addressed

We delivered a fluorescently labelled murine pancreatic cancer cell line (KPC-ZsGreen), intravenously into mice, alongside healthy controls for comparison. Lungs were harvested two days post-injection, and neutrophils isolated from the bone marrow and spleen were labelled with CellTrace Yellow before being added to lung slices. Pre-existing tissue endogenous neutrophils were labelled with anti-Ly6G AF647. After a 20-hour incubation, live imaging with Zeiss LSM 880 confocal microscope captured 3D Z-stack time-lapse videos

over 40 minutes (Figure 1a, b). Imaging data were analysed in Imaris, and neutrophil motility metrics—including speed, track straightness, arrest coefficient, tumour approach speed, and clustering—were quantified in R.

In addition, I constructed an agent-based mathematical model to stimulate neutrophil behaviours. The positions of the neutrophils are governed by random walk dynamics, with a bias towards the tumour, a tendency for clustering and a probability of arrest (see equation below).

$$X_{-}(t+1,i)=X_{-}(t,i)+m_{-}(t,i) v_{-}(t,i) s_{-}mult \hat{v}_{-}(t,x,i) \Delta t$$

$$Y_{-}(t+1,i)=Y_{-}(t,i)+m_{-}(t,i) v_{-}(t,i) s_{-}mult \hat{v}_{-}(t,y,i) \Delta t$$

Where $X_{-}i$ and $Y_{-}i$ are the x and y coordinates of the neutrophils $i=1,2,\dots,200$ respectively at time t . Simulation performed on a 500 by 500 square domain, with fixed timestep (duration of each movement) Δt . At each timestep, neutrophils migrate with probability $m_{-}i$ and speed $v_{-}i$. $s_{-}mult$ is the speed multiplier, $\hat{v}_{-}(x,i)$ is the direction of movement.

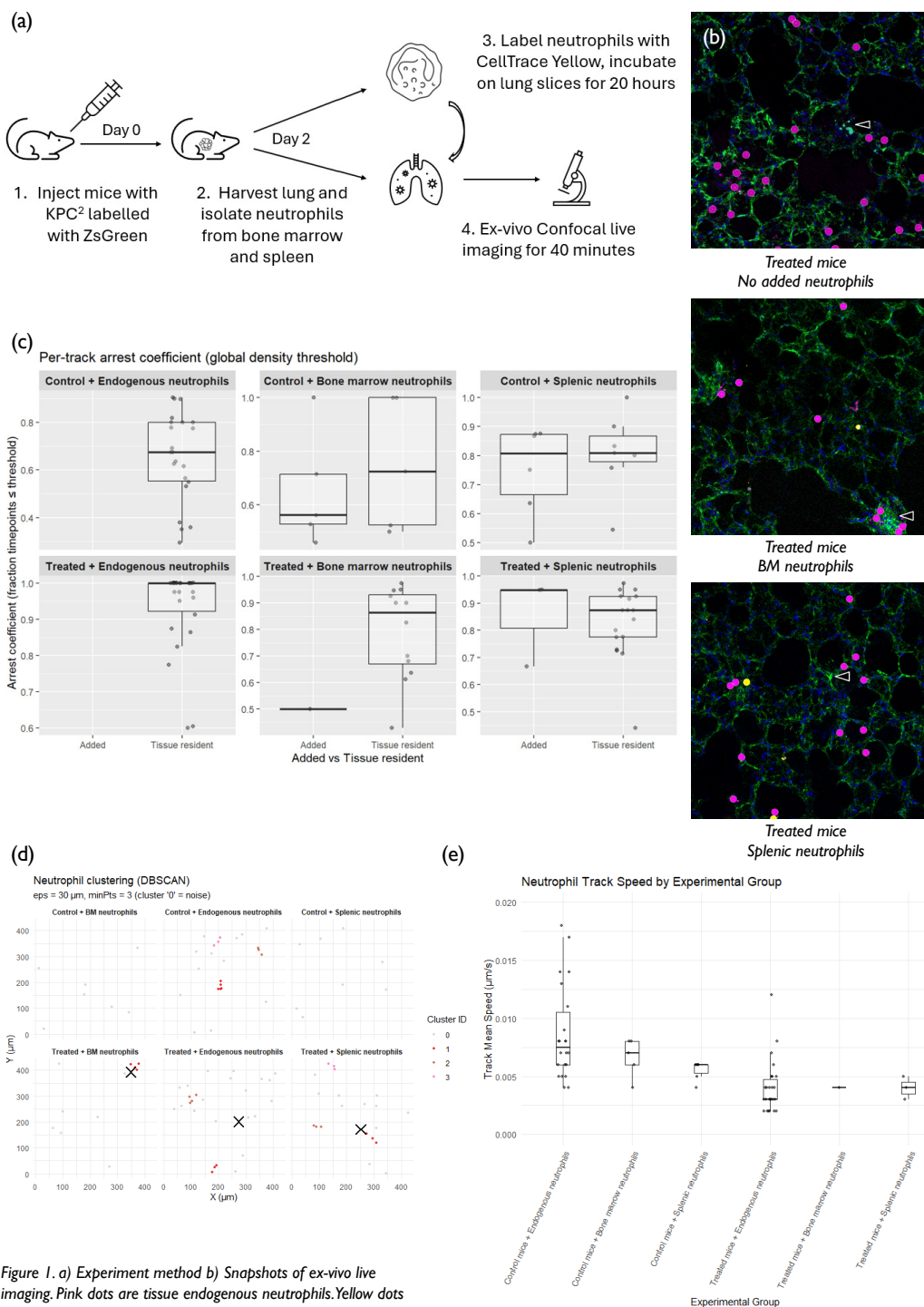


Figure 1. a) Experiment method b) Snapshots of ex-vivo live imaging. Pink dots are tissue endogenous neutrophils. Yellow dots are added bone marrow/splenic neutrophils. Black arrows point to clusters of tumour cells. Hoechst – Cell nucleus; ZsGreen – KPC; AF488 – Vasculature; CellTrace Yellow – Added neutrophils; AF647 – Endogenous neutrophils. c) Arrest coefficients of neutrophil tracks, per experimental group. Arrest threshold $< 0.005 \mu$ m. d) Neutrophil clustering analysis by Density-based spatial clustering of applications

with noise (DBSCAN) algorithm. Radius of swarm size = 30 μ m, minimum neighbours to form cluster = 3. Black cross shows position of metastasis. e) Neutrophil track speed by experimental group.

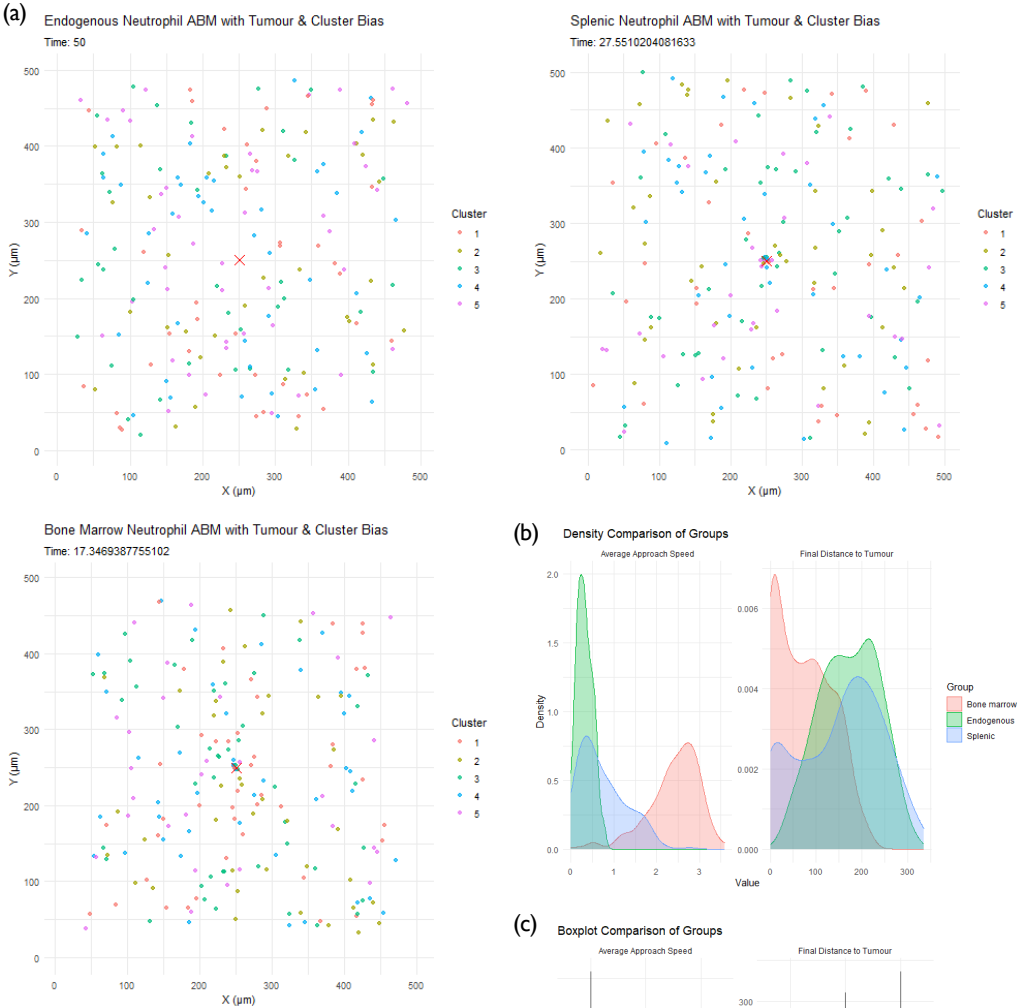


Figure 2. a) Snapshots of agent based model. b) density plot of average tumour approach speed and distance to tumour at $t = 50$, per group. c) boxplot summary of average tumour approach speed and distance to tumour at $t = 50$, per group.

Findings

My data showed no significant difference in mean track speed or straightness between bone marrow- and spleen-derived neutrophils in either control or tumour-bearing mice (Figure 1e). However, due to technical limitations we couldn't address in the time of the project, only I added bone marrow derived neutrophil was identified, limiting statistical power. Both bone marrow and spleen neutrophils migrated towards tumour cells, and formed clusters around sites of metastasis (Figure 1d). With the spleen-

derived population we were able to track, many cells displayed high arrest coefficients, and slowed down noticeably as they approached tumours (Figure 1c). These behaviours might indicate trafficking of neutrophils toward the tumour. Our data suggests that further studies into whether the site of neutrophil production, bone marrow versus spleen, would affect their behaviour in the metastatic lung environment, are required.

I generated an agent-based model to complement the imaging data and potentially overcome some of the experimental limitations we faced due to the limited number of experiments we could achieve in the project – such as the low number of observable neutrophils, reduced motility over time and limited neutrophil tissue infiltration. In the $500 \times 500 \mu\text{m}$ simulated tissue slice, 200 neutrophils were initiated at randomised positions, with speed and arrest probability inferred from regression models constructed using experimental measurements as a function of distance to the tumour. The model recapitulated the migration of both bone marrow- and spleen-derived neutrophils toward tumours and the formation of clusters (Figure 2a). Importantly, even subtle differences in behaviour could produce measurable changes in tumour engagement over time. When parameters for spleen-derived neutrophils were applied, simulated cells exhibited dynamics resembling those of tissue-resident neutrophils, raising the possibility that spleen-derived neutrophils may contribute disproportionately in number to immune cell accumulation around early metastatic foci. This is reflected in the similarity of the final distance to tumours between splenic and endogenous neutrophils (Figure 2b, c). Lastly, the ABM enabled exploration of dynamics beyond the experimental timeframe, circumventing photobleaching and other limitations inherent to prolonged live imaging.

What I learned from participating in this project

This project gave me hands-on experience in microscopy, live imaging, mouse work, tissue and cell culture. I gained practical skills in operating a high-resolution confocal microscope and analysing complex imaging data. Particularly, I realised the effort required to optimise experimental systems. Working closely with experienced researchers also taught me problem-solving strategies. For example, we used fixed imaging to determine the optimal incubation time for neutrophils to infiltrate lung tissue slices. The chance to see immune-tumour

interaction in real time was a highlight of the project and finally I could truly appreciate the power of microscopy to uncover cellular behaviour that was only taught to me in classrooms. Lastly, I used my mathematical modelling skills that I developed previously to complement my imaging analysis. This underscored the importance of multi-disciplinary collaboration in scientific research.

Impact on long-term goals

Participating in this project has strengthened my interest in cancer immunology and imaging-based research. I plan to intercalate a masters degree in between my preclinical and clinical years of my medical school. I believe the skills I have gained will help me become a more informed and effective clinician, able to critically evaluate new research and apply insights from basic science to patient care. I realised that scientific research and clinical implementation are complementary to each other. Gaining a strong foundation in preclinical methods such as microscopy and computational modelling will allow me to engage more deeply with future developments in oncology and immunology, ultimately benefiting my patients by bridging the gap between bench and bedside.



Shang Wang.