

# Zinc isotopic compositions of breast cancer tissue

## Electronic Supplementary Information

Fiona Larner<sup>\*a,b</sup>, Laura N. Woodley<sup>c</sup>, Sami Shousha<sup>d</sup>, Ashley Moyes<sup>e</sup>,  
Emma Humphreys-Williams<sup>f</sup>, Stanislav Strekopytov<sup>f</sup>, Alex N. Halliday<sup>a</sup>,  
Mark Rehkämper<sup>b,f</sup>, R. Charles Coombes<sup>g</sup>

<sup>a</sup>Department of Earth Sciences, University of Oxford, South Parks Road, Oxford, OX1 3AN, UK

<sup>b</sup>Department of Earth Science & Engineering, Imperial College London, Exhibition Road, South Kensington, London, SW7 2AZ, UK

<sup>c</sup>Experimental Cancer Medicine Centre Network, Imperial College, Fulham Palace Road, London W6 8RF, UK

<sup>d</sup>Department of Histopathology, Charing Cross Hospital, Imperial College, Fulham Palace Road, London W6 8RF, UK

<sup>e</sup>Medical Oncology, Charing Cross Hospital, Imperial College NHS Trust, Fulham Palace Road, London W6 8RF, UK

<sup>f</sup>Department of Earth Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

<sup>g</sup>Department of Surgery and Cancer, Imperial College London, Du Cane Road, London, W12 0NN, UK

\*corresponding author: Dr Fiona Larner, Department of Earth Sciences, University of Oxford, South Parks Road, Oxford, OX1 3AN, UK. +44 (0)1865 282117; [fiona.larner@earth.ox.ac.uk](mailto:fiona.larner@earth.ox.ac.uk)

### ***Isotope Mixing Calculations***

The isotope composition of a pool, which is formed by mixing of two separate reservoirs (denoted as A and B), is defined by:

$$\delta^{66}\text{Zn}_C = (\delta^{66}\text{Zn}_A \times F_A) + (\delta^{66}\text{Zn}_B \times F_B) \quad (\text{S1})$$

where C denotes the mixture of the two pools,  $\delta^{66}\text{Zn}$  is the isotopic composition of the reservoirs, and  $F_X$  stands for the molar fraction of Zn in reservoir A or B.

As  $F_A + F_B = 1$ , equation S1 can be simplified to:

$$\delta^{66}\text{Zn}_C = (\delta^{66}\text{Zn}_A \times F_A) + (\delta^{66}\text{Zn}_B \times (1 - F_A)) \quad (\text{S2})$$

A change or fractionation in isotopic composition is reported relative to the starting composition by:

$$\Delta^{66}\text{Zn} = \delta^{66}\text{Zn}_C - \delta^{66}\text{Zn}_A \quad (\text{S3})$$

Equations S1 and S2 can be rearranged to determine the isotopic composition that results for a reservoir, if some fraction of Zn with a distinct isotope composition is sequestered from this pool. In our case, Zn is taken up by the tumour from the blood ( $\delta^{66}\text{Zn}_{\text{in}}$ ), sequestered within the tumour ( $\delta^{66}\text{Zn}_{\text{seq}}$ ), and the tumour also expels residual 'tumour-derived' Zn ( $\delta^{66}\text{Zn}_{\text{out}}$ ):

$$\delta^{66}\text{Zn}_{\text{out}} = (\delta^{66}\text{Zn}_{\text{in}} - (\delta^{66}\text{Zn}_{\text{seq}} \times F_{\text{seq}})) / (1 - F_{\text{seq}}) \quad (\text{S4})$$

As an additional complication, the initial uptake of Zn from blood into tumorous cells may occur either with or without isotope fractionation  $\Delta^{66}\text{Zn}_{\text{uptake}}$ .

$$\Delta^{66}\text{Zn}_{\text{uptake}} = \delta^{66}\text{Zn}_{\text{in}} - \delta^{66}\text{Zn}_{\text{blood}} \quad (\text{S5})$$

If there is no isotope fractionation during Zn uptake, then  $\Delta^{66}\text{Zn}_{\text{uptake}} = 0$ , so that  $\delta^{66}\text{Zn}_{\text{in}} = \delta^{66}\text{Zn}_{\text{blood}} \approx +0.1 \text{ ‰}$ .

If Zn isotope fractionation occurs during uptake, the heavy isotopes of Zn are likely transferred into the cells preferentially (see text), so that  $\Delta^{66}\text{Zn}_{\text{uptake}} > 0$ . This implies that  $\delta^{66}\text{Zn}_{\text{in}} > +0.1 \text{ ‰}$ .

Once released, tumour-derived Zn (with  $\delta^{66}\text{Zn}_{\text{out}}$ ) is subsequently mixed with the Zn already present in the reservoir to which it is expelled (e.g., white blood cells,  $\delta^{66}\text{Zn}_{\text{res}}$ ). The isotopic composition of the resulting mixture  $\delta^{66}\text{Zn}_{\text{mix}}$  can be determined by applying equation S1:

$$\delta^{66}\text{Zn}_{\text{mix}} = (\delta^{66}\text{Zn}_{\text{out}} \times F_{\text{out}}) + (\delta^{66}\text{Zn}_{\text{res}} \times F_{\text{res}}) \quad (\text{S6})$$

The addition of tumour-derived Zn thus produces a change in isotope composition for this reservoir, denoted  $\Delta^{66}\text{Zn}_{\text{mix}}$

$$\Delta^{66}\text{Zn}_{\text{mix}} = \delta^{66}\text{Zn}_{\text{mix}} - \delta^{66}\text{Zn}_{\text{res}} \quad (\text{S7})$$

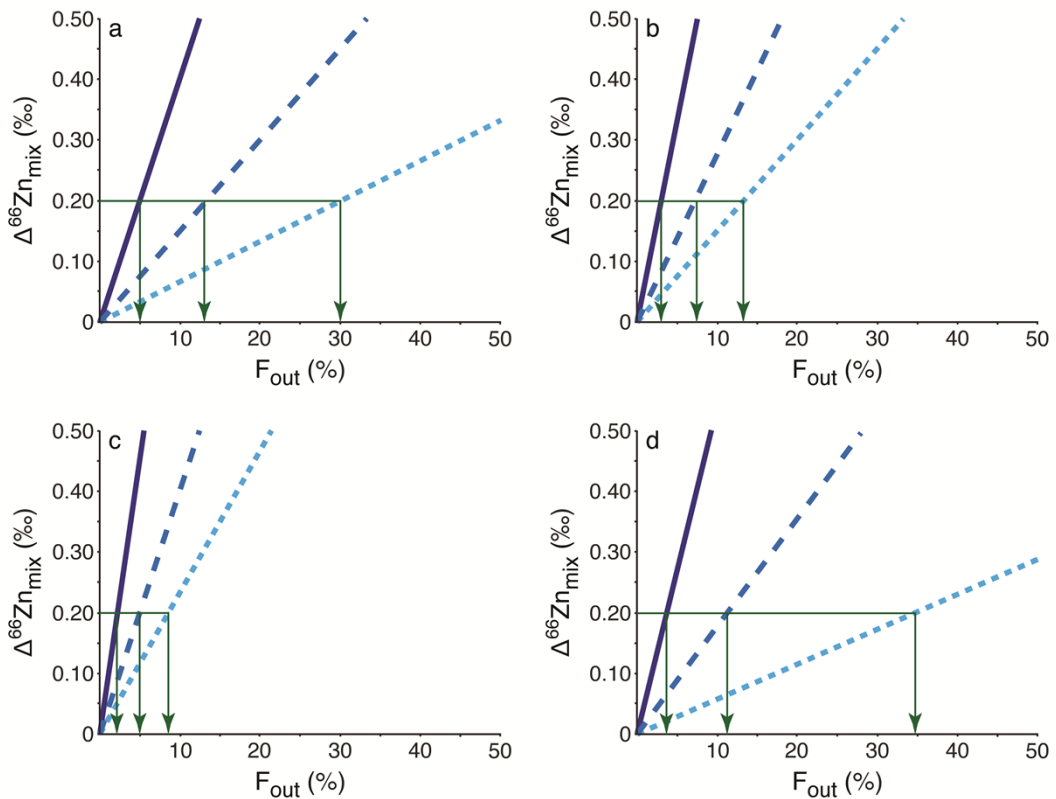
Detection of tumour-derived Zn in this pool then requires that the change in isotopic composition  $\Delta^{66}\text{Zn}_{\text{mix}}$ , exceeds the precision of the analytical method. In our case, this analytical threshold is conservatively estimated to be  $\pm 0.20$  ‰.

A plot of  $\Delta^{66}\text{Zn}_{\text{mix}}$  relative to  $F_{\text{out}}$  can be used to determine the minimum value of  $F_{\text{out}}$  that is required, so that the expelled ‘tumour-derived’ Zn may be detected in the diagnostic pool.

In our case, the mass balance of the system is only poorly constrained. Therefore, we calculate  $\Delta^{66}\text{Zn}_{\text{mix}}$  (using equations S4 to S7) by considering a reasonable range of values for (i) the fraction of Zn sequestered by the tumour,  $F_{\text{seq}}$  (equation S4), (ii) the Zn isotope fractionation  $\Delta^{66}\text{Zn}_{\text{uptake}}$  that is associated with Zn uptake by the tumour cells prior to sequestration (equation S5), and (iii) the initial isotope composition  $\delta^{66}\text{Zn}_{\text{res}}$  of the diagnostic pool (equations S6, S7). The results of these model calculations are summarized in Fig. S1.

In detail, the  $\delta^{66}\text{Zn}$  for Zn in blood and sequestered in tumours is constrained by our analytical results (Fig. 1, main text) and values of  $+0.1$  ‰ and  $-0.9$  ‰, respectively, were used for the modelling. The isotope fractionation  $\Delta^{66}\text{Zn}_{\text{uptake}}$  for transfer of Zn from blood to tumour cells (before sequestration) was varied between  $0$  ‰ and  $+1$  ‰. The (molar) fraction of Zn sequestered by the tumour ( $F_{\text{seq}}$ ) was assumed to be between  $40$  % and  $80$  %. Finally, values of  $+0.1$  ‰ (equivalent  $\delta^{66}\text{Zn}_{\text{blood}}$ ) and  $+1$  ‰ were chosen for the initial isotope composition  $\delta^{66}\text{Zn}_{\text{res}}$  of the diagnostic pool.

Depending on the combination of these factors, about  $3$  to  $35$ % of the Zn in the diagnostic pool must be tumour-derived Zn to enable detection (Fig. S1).



**Figure S1. Estimated diagnostic sensitivity based on available isotopic constraints.** Tumour-derived Zn is detectable in a diagnostic pool if this generates an isotopic difference  $\Delta^{66}\text{Zn}_{\text{mix}}$  exceeding  $\pm 0.2$  ‰ (green lines). This condition is achieved if the (molar) fraction of tumour-derived Zn present in the diagnostic pool ( $F_{\text{out}}$ ) is equivalent to about 3 to 35%.

All scenarios assume that the tumour sequesters 40 % (dotted line), 60 % (dashed line) or 80% (solid line) of the input Zn flux, and the sequestered Zn is characterized by  $\delta^{66}\text{Zn}_{\text{seq}} = -0.9$ ‰. Cases **A**, **B** and **C** presume that the original Zn isotope composition of the diagnostic pool (prior to addition of tumour-derived Zn) is identical to blood and serum, so that  $\delta^{66}\text{Zn}_{\text{res}} = +0.1$ ‰. In this case  $\Delta^{66}\text{Zn}_{\text{mix}} = \delta^{66}\text{Zn}_{\text{mix}} - 0.1$ ‰ (see equation S7). No isotope fractionation occurs during Zn transfer to the tumour cells in Case **A** ( $\Delta^{66}\text{Zn}_{\text{uptake}} = 0$ ; equation S5). Isotope fractionation during initial Zn uptake by the tumour occurs for Case **B** ( $\Delta^{66}\text{Zn}_{\text{uptake}} = +0.5$ ‰) and **C** ( $\Delta^{66}\text{Zn}_{\text{uptake}} = +1$ ‰). Case **D** assumes  $\delta^{66}\text{Zn}_{\text{res}} = +1$ ‰, and  $\Delta^{66}\text{Zn}_{\text{uptake}} = +0.5$ ‰.