Zinc isotopic compositions of breast cancer tissue

Electronic Supplementary Information

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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:

$$\partial^{66}Zn_C = (\partial^{66}Zn_A \times F_A) + (\partial^{66}Zn_B \times F_B) \quad \text{(S1)}$$

where C denotes the mixture of the two pools, $\partial^{66}Zn$ 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:

$$\partial^{66}Zn_C = (\partial^{66}Zn_A \times F_A) + (\partial^{66}Zn_B \times (1 - F_A)) \quad \text{(S2)}$$

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

$$\Delta^{66}Zn = \partial^{66}Zn_C - \partial^{66}Zn_A \quad \text{(S3)}$$

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 ($\partial^{66}Zn_{in}$), sequestered within the tumour ($\partial^{66}Zn_{seq}$), and the tumour also expels residual 'tumour-derived' Zn ($\partial^{66}Zn_{out}$):

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

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

$$\Delta^{66}Zn_{uptake} = \partial^{66}Zn_{in} - \partial^{66}Zn_{blood} \quad \text{(S5)}$$

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

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

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

$$\partial^{66}Zn_{mix} = (\partial^{66}Zn_{out} \times F_{out}) + (\partial^{66}Zn_{res} \times F_{res}) \quad \text{(S6)}$$

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

$$\Delta^{66}Zn_{mix} = \partial^{66}Zn_{mix} - \partial^{66}Zn_{res} \quad \text{(S7)}$$

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

A plot of $\Delta^{66}Zn_{mix}$ relative to F_{out} can be used to determine the minimum value of F_{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 $\Delta^{66}Zn_{mix}$ (using equations S4 to S7) by considering a reasonable range of values for (i) the fraction of Zn sequestered by the tumour, F_{seq} (equation S4), (ii) the Zn isotope fractionation $\Delta^{66}Zn_{uptake}$ that is associated with Zn uptake by the tumour cells prior to sequestration (equation S5), and (iii) the initial isotope composition $\partial^{66}Zn_{res}$ of the diagnostic pool (equations S6, S7). The results of these model calculations are summarized in Fig. S1.

In detail, the $\partial^{66}Zn$ 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 $\Delta^{66}Zn_{uptake}$ 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_{seq}) was assumed to be between 40 % and 80 %. Finally, values of +0.1 ‰ (equivalent $\partial^{66}Zn_{blood}$) and +1 ‰ were chosen for the initial isotope composition $\partial^{66}Zn_{res}$ 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).

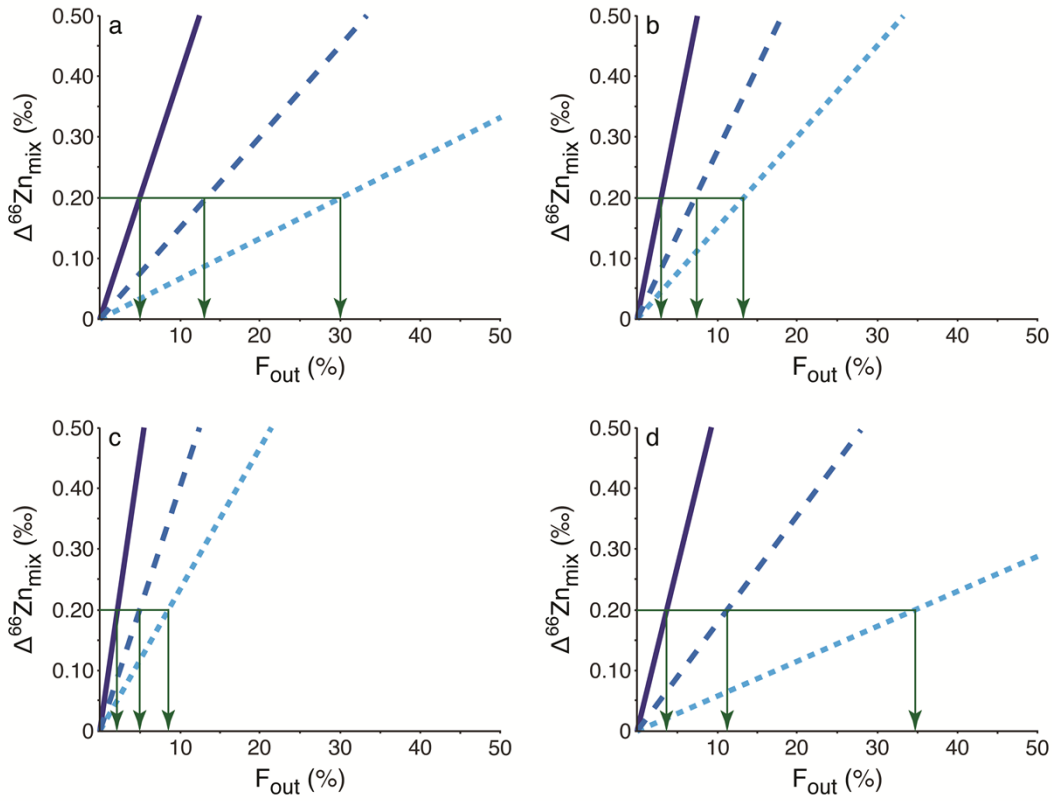

Figure S1. Estimated diagnostic sensitivity based on available isotopic constraints. Tumour-derived Zn is detectable in a diagnostic pool if this generates an isotopic difference $\Delta^{66}Zn_{mix}$ exceeding ±0.2 ‰ (green lines). This condition is achieved if the (molar) fraction of tumour-derived Zn present in the diagnostic pool (F_{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 $\partial^{66}Zn_{seq}$ = –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 $\partial^{66}Zn_{res}$ = +0.1 ‰. In this case $\Delta^{66}Zn_{mix}$ = $\partial^{66}Zn_{mix}$ – 0.1 ‰ (see equation S7). No isotope fractionation occurs during Zn transfer to the tumour cells in Case **A** ($\Delta^{66}Zn_{uptake}$ = 0; equation S5). Isotope fractionation during initial Zn uptake by the tumour occurs for Case **B** ($\Delta^{66}Zn_{uptake}$ = +0.5 ‰) and **C** ($\Delta^{66}Zn_{uptake}$ = +1 ‰). Case **D** assumes $\partial^{66}Zn_{res}$ = +1 ‰, and $\Delta^{66}Zn_{uptake}$ = +0.5 ‰.