# **Supplementary material**

# **1.** Rough worked example - Predicting enrichment of labelled cells to align with analytic capabilities

A rough worked example can be made to estimate impact of dosing on measured deuterium enrichments of DNA in labelled cells.

### Input parameters:

- a. Estimated proliferation rate of cells of interest
- b. Rate of administration of deuterated water
- c. The analytic range and precision of the mass spectrometer used

### Assumptions

- a. Body water volume ~42L for adult male adjust for other categories of participant
- b. Body water turnover  $\sim$  3L/day a behavioural variable
- c. The number of labelled deuterium atoms per analysed moiety, or 'scaling fator' (bw or c) see Discussion

## Suppose:

- (i) we wish to study a cell whose proliferation rate is expected to be about 1% per day;
- (ii) we give deuterated water as shown in **Figure 1** at a rate of 200 ml of 70% on day one, followed by 100 ml of 70% daily thereafter.

In this case, we will achieve deuterium enrichments in body water of about 1-2% APE over the course of the labelling phase – although modelled dynamically let us take a constant value of 1.5% for the sake of this approximate illustration.

For a cell proliferating at about 1%/day, after 1 week ~7% of cells will be 'new'.

Each new cell will be labelled according to {the proportion of deuterium-enrichment in all hydrogens (~1.5% or x ~0.015)} x {the number of labelled deuterium atoms per analysed moiety (~5)}, so the expected enrichments in DNA will be:

The proportion of 'new' cells,  $0.07 \ge 0.015 \ge 5 = 0.005$  or 0.5% APE

(iii) we have a mass spectrometer which measures deuterium enrichment with a typical standard deviation of replicates of 0.05 atoms percent enrichment (APE)

On analysis the expected value for deuterium enrichment then represents a z-score of 10 (0.5 / 0.05) so should be readily measurable with a high degree of accuracy.

At 7 weeks, because some new cells are lost, the proportion of new cells will be ~39%, so predicted labelling is  $0.39 \ge 0.015 \ge 2.9\%$ , well in the measurement range.

#### 2. In silico predictions of enrichment of labelled cells

More accurate predictions can be made *in silico* by modelling that takes into account the dynamics of deuterated water and cell labelling. This would be achieved in the following manner:

Construct a model to describe label in your cell population of interest or use an existing model (equation 3 in <sup>1</sup> is a generic model suitable for many cell populations). Reproduced here for ease of reference:

$$\dot{L} = pb_{w}S(t) - d^{*}L$$

Where

$$f(1 - e^{-\delta t}) \qquad t \le \tau$$
  
$$S(t) = \qquad f(1 - e^{-\delta \tau})e^{-\delta(t - \tau)} \qquad \tau < t$$

Substitute in realistic numerical values (for example for this model the parameters bw, f and  $\delta$ are all tabulated in Supplementary Table 3 in <sup>1</sup>, medians could be used). By definition the kinetic parameters of the cell population (p and  $d^*$ ) are unlikely to be known but a range of plausible guesses can be made, in turn, spanning the range where the true value is thought to lie. For each of these values of p and  $d^*$ , substituting them into the equations and solving will give a predicted value of label over time for a given choice of labelling period  $\tau$ . Points can then be "sampled" at times that reflect the sampling strategy of interest, noise added (a random variable from a normal distribution with mean zero), and added to the "sampled label". In this way an *in silico* data set (for known p and  $d^*$ ) can be generated. From here, you follow the proposed modelling protocol as if this was your observed data, fitting the choice of model (not necessarily the generating model) to the *in silico* data and the parameters estimated, giving  $\hat{p}$  and  $\hat{d}^*$ . The discrepancy between the true (generating) values of p and  $d^*$  and their estimates  $\hat{p}$  and  $\hat{d}^*$  can be assessed. If the errors are too large then the experimental protocol will need to be altered – perhaps a different length of labelling period or a larger number of samples, until the level of error is considered acceptable. This process will then need to be repeated for all values of p and  $d^*$  that span the potential range.

1. Zhang, Y. *et al.* KIR-HLA interactions extend human CD8+ T cell lifespan in vivo. *J. Clin. Invest.* **133** (2023).