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An extended *ex vivo* HSC expansion platform enables rapid, multiplex in vivo functional genomics for lymphoma

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Current experimental models for functional genomics in lymphoma each have important limitations. *In vitro* systems fail to capture the important contribution of the immune microenvironment, while transgenic mouse models are slow, costly, and typically limited to perturbing small numbers of genes in combination. *Ex vivo* engineering of murine haematopoietic stem cells (HSCs) followed by transplantation offers a flexible alternative, but has historically been constrained by rapid loss of stem cell potential during in vitro culture.

We leveraged recently developed methods for extended *ex vivo* expansion of murine HSCs, enabling culture for up to six weeks without loss of repopulating capacity. This window facilitates efficient genetic modification and selection of cells carrying multiple simultaneous genetic perturbations. Following transplantation into lethally irradiated recipients, engineered HSCs reconstitute a haematopoietic compartment supporting both transgene overexpression and CRISPR-based gene knockout (Fig.1A).

To achieve lineage-restricted oncogene activation, we combined HSCs from Cre-transgenic donors with viral vectors incorporating a LoxP-dependent flip-excision cassette, enabling activation of wild-type or mutant oncogenes specifically in the B-cell lineage or within the germinal centre (Fig1B). The platform is readily integrated with established transgenic backgrounds, including Cas9 and oncogene-transgenic mice, to accelerate generation of genetically tailored lymphomas in a fully functional immune microenvironment.

We present examples demonstrating rapid production of customised tumours *in vivo* and show that the approach is scalable for pooled screening, including simultaneous evaluation of multiple wild-type and mutant open reading frames and CRISPR-mediated deletion of candidate tumour suppressors. This experimentally agile system enables modelling of lymphoma genetic complexity while preserving physiological tumour-immune interactions.

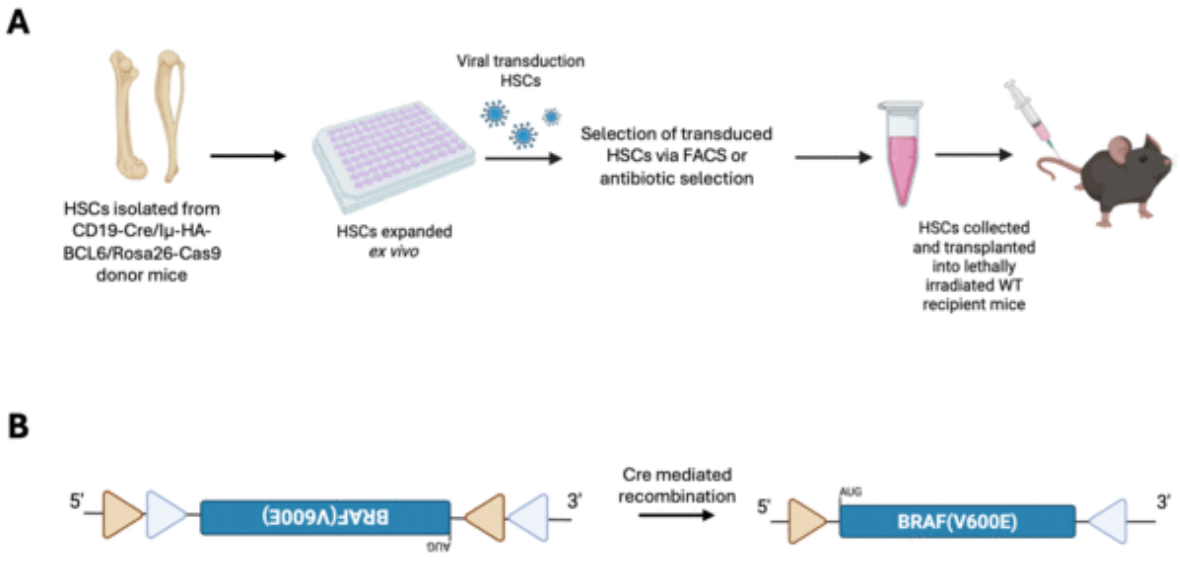

Figure 1. HSC transplant protocol, and flip-excision method of conditional gene expression
(A) Schematic representing the protocol to transplant genetically modified haematopoietic stem cells *in vivo*. (B) Diagram showing the flip-excision construct used to achieve conditional Cre-dependent gene expression, using mutant *BRAF(V600E)* as an example.