CRISPR KO in HSPCs to evaluate hemetox target safety liabilities: proof of concept

Jasper Komen[1], Benedicte Recolin[1], Giovanni Ciotta[2], Anne Goeppert[3], Muireann Coen[1], Clay Scott[1]

[1]Oncology Safety, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Cambridge UK
[2]Discovery Biology, Discovery Sciences, R&D, AstraZeneca, Cambridge UK
[3]Precision Medicine, Oncology, R&D, AstraZeneca, Cambridge, UK

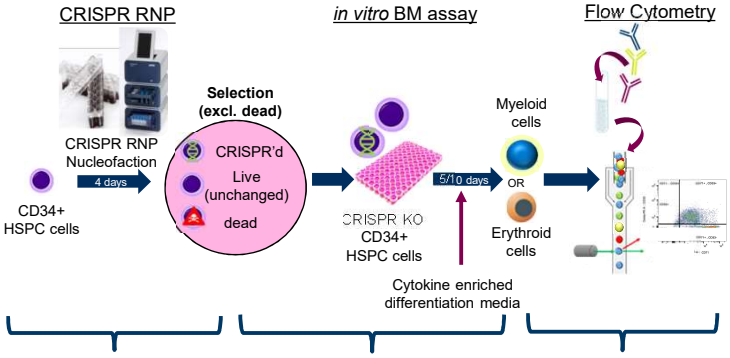
Introduction

- Understanding the role of oncology drug targets in hematopoietic stem cells is very important as side-effects in the bone marrow (BM) often limit the therapeutic utility of anti-cancer agents.
- Our current 2D BM endpoint viability assay uses human CD34+ hematopoietic stem/progenitor cells (HSPCs) driven to differentiate into erythroid, myeloid, or megakaryocytic cell-lineages using specialist liquid culture medium allowing a higher throughput of BM toxicity (hemetox) assessment than the standard Colony Forming Unit (CFU) assay.
- Unfortunately, none of the available *in vitro* assays can discriminate between on- or off-target safety liabilities.
- Here, we describe the generation and implementation of CRISPR KO mutant HSPCs for evaluation of on-target BM safety risks using the non-essential *PTPRC* gene (encoding CD45) as proof of concept (POC).

Aim

Demonstrate POC for using CRISPR RNP to generate KO HPSCs for evaluation of hemetox safety issues related to target biology.

Methods



1) Cas9:guide RNA ribonuclear protein (RNP) was transfected into expanded CD34+ HSPCs (4d.) by nucleofection (electroporation). CRISPR induced DNA changes were measured by Tracking of Indels by Decomposition (TIDE) analysis.

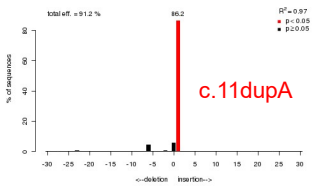
2) Day 4 post-transfection viable cells are selected by Magnetic-Activated Cell Sorting (MACS) dead cell removal kit. The live CRISPR KO CD34+ cells are grown in lineage-specific differentiation media.*

3) Cells are stained for CD45 (target), progenitor cells or cells of erythroid, myeloid lineages to identify differentiation stage by flow cytometry (FC).

*erythroid: Human P-BFU1; myeloid: Human P-GM1 (both CellExpand)

Results

A) DNA changes in CD45 by TIDE



B) CD45 expression by FC

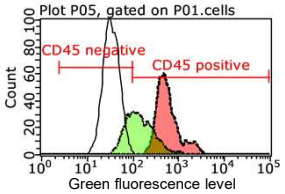


Figure 1. CRISPR RNP* efficiently introduces DNA changes resulting in decreased CD45 protein expression in CD34+ HSPCs. DNA changes in CD34+ HSPCs (4d. Post-transfection) showed >90% efficiency (TIDE analysis) **(A)** and >80% decrease in CD45 protein level (FC) in KO vs WT **(B)**. Predominant change was c.11dupA resulting in premature stop codon in exon 1.
*Multiple guide RNAs designed in house and tested to get optimal efficiency

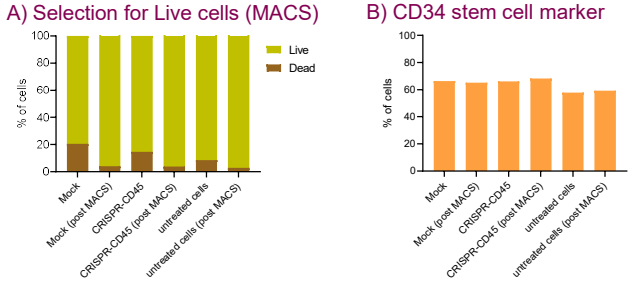


Figure 2. Cell death is similar in CRISPR RNP and mock nucleofected samples whilst MACS selected live HSPCs maintain stem cell marker CD34. PI staining of dead cells (A), and presence of CD34 by FC (B) on day 4 post-transfection. Live cell enrichment did not change the level of CD45 DNA changes and reduction of CD45 protein (data not shown).

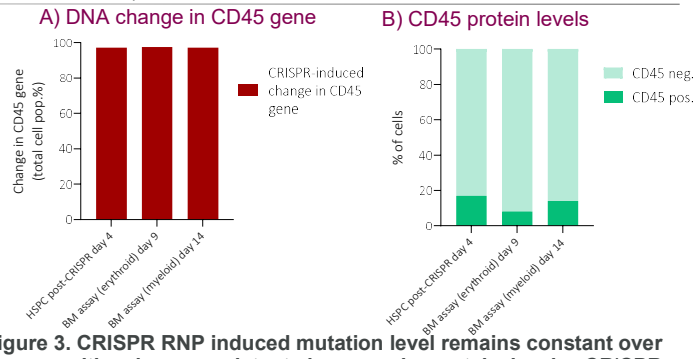


Figure 3. CRISPR RNP induced mutation level remains constant over time resulting in a consistent decrease in protein levels. CRISPR induced DNA changes in the *CD45* gene by TIDE in the different cell population over time (A). CD45 protein levels detected in the same samples by FC (B).

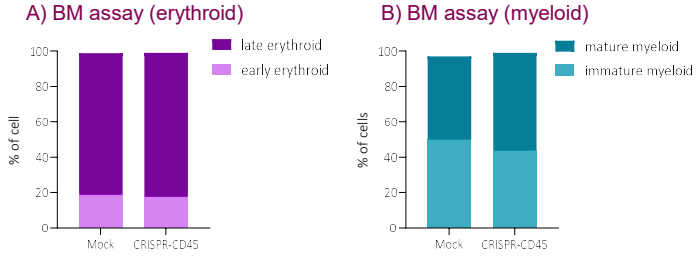


Figure 4. CRISPR induced CD45 KO causes no change in BM differentiation as expected[1,2,3]. Early (CD71+/CD235-) and late (CD71+/CD235+) erythroid cells detected by flow cytometry after 5 day BM assay (erythroid) (A). Immature (CD33+/CD15-) and mature (CD33+/CD15+) myeloid cells detected by FC after 10 day BM assay (myeloid) (B).

Conclusions

- Highly robust and efficient CRISPR KO of CD45 in HSPCs demonstrates the feasibility for application in hemetox target safety assessment.
- The CD45 KO cells retained the capability to differentiate into the erythroid and myeloid lineages in our 2D bone marrow (BM) assay as anticipated[1,2,3].
- The knockout of a lineage specific gene (e.g. EPO-R) as positive control will identify the exact window of detection for assessment of drug targets by CRISPR KO in our 2D Bone Marrow assay.
- Discussions are currently underway for targets in the early-oncology portfolio to help clarify their role in stem cell proliferation and differentiation into various hematopoietic lineages.

References
1. Irie-Sasaki et al., 2001, Nature 409, 349-54
2. Harashima et al., 2002, Blood 100, 4440-4445
3. Gundry et al., 2016, Cell Reports 17, 1453–1461

Supported by AstraZeneca

Presented at the CRISPR in Drug Discovery 2021 (ELRIG), 23-24 March