# A novel circulating tumor DNA (ctDNA) assay enables monitoring of disease progression and treatment response in disseminated preclinical hematologic cancer models

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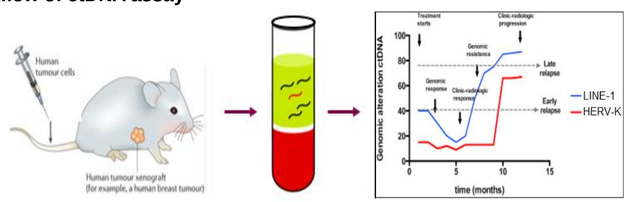
#2957

## Introduction

- Disseminated models of hematologic cancers are biologically relevant but pose logistical hurdles in disease monitoring.
- Assays such as flow cytometry, bioluminescence imaging (BLI), and histology are limited by sensitivity of detection – can't reliably detect minimal residual disease (MRD).
- Current methods for staging often require satellite mice or procedures involving anesthesia (e.g. BLI or live bone marrow aspirates) which conflicts with the 3Rs of animal welfare.
- **We developed a human-specific ddPCR assay using multicopy genetic targets to detect ctDNA in mouse plasma with subcellular sensitivity.**

## Development of ctDNA assay

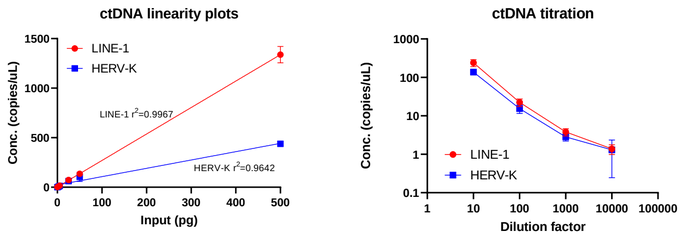
### A. Workflow of ctDNA assay




***LINE-1*: human transposable element represents ~17% of the human genome (MORE sensitive – used for early disease detection)**

***HERV-K*: human endogenous retrovirus (LESS sensitive – used for late-stage disease states to prevent assay saturation)**

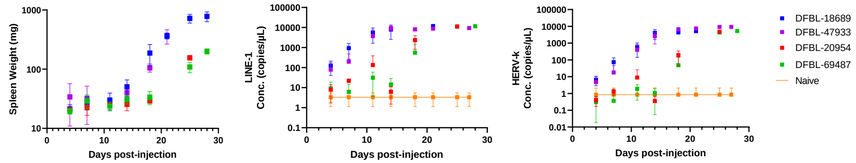
### B. Assay validation



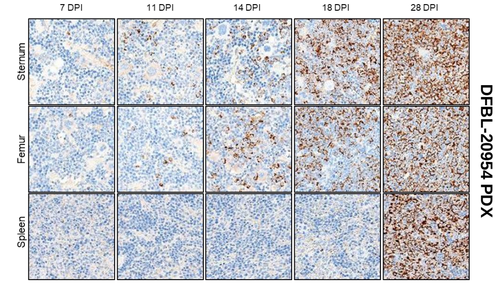

- A linearity experiment identified a minimum DNA input of ~2.7pg (0.8 haploid genome equivalent) to generate consistent data between 3 replicates. This led us to determine that 40uL of blood was optimal to minimize blood collection while optimizing assay performance.
- Serial dilution of diseased mouse plasma (human acute myeloid leukemia) with naïve plasma confirmed that the assay was sensitive enough to accurately detect ctDNA over several orders of magnitude.

## ctDNA levels track with disease burden in multiple disseminated hemes models

### C. ctDNA tracking in DLBCL models

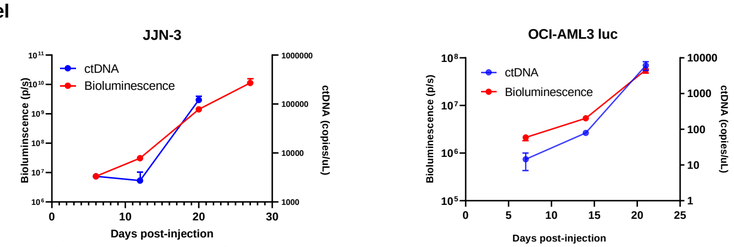


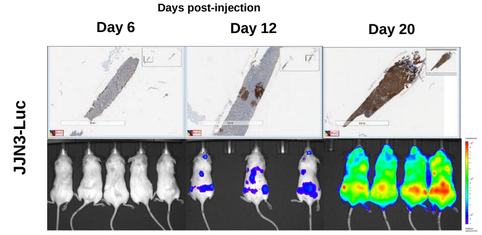

| Model | Subtype |
|---|---|
| DFBL-18689 | ABC |
| DFBL-47933 | ABC |
| DFBL-20954 | DHL-GCB |
| DFBL-69487 | DHL-GCB |




- Disease progression in diffuse large B-cell lymphoma (DLBCL) PDX models was monitored longitudinally by spleen weight and IHC for a human-specific marker (huMitoTracker).
- Progression of ctDNA levels tracked with disease progression in bone marrow and spleen.
- Models that engraft early in soft tissues have higher early levels of ctDNA; however, even models with no increase in spleen weight have ctDNA above baseline only 4 days post-injection.

### D. Correlation between ctDNA & BLI in JJN-3 multiple myeloma and OCI-AML3 leukemia model

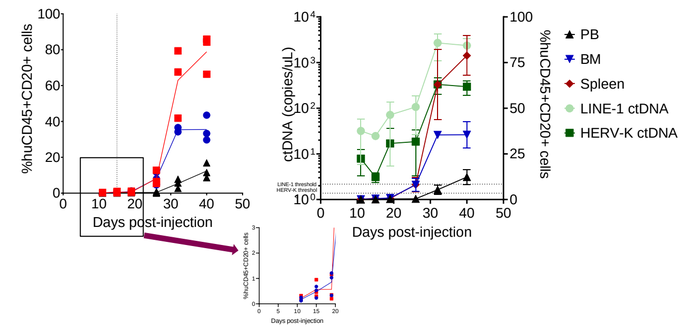



- ctDNA and BLI disease progression were similar across both models tested, though at first timepoint disease was already significantly above baseline.
- IHC confirms JJN3-Luc disease is progressing similarly in the femur using huMitoTracker.

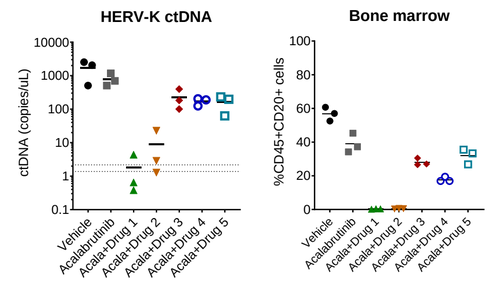
## ctDNA mirrors pre- and post-treatment disease in disseminated MCL PDX

### E. Monitoring disease burden in untreated MCL PDX-bearing mice




- Mice were injected intravenously with a mantle cell lymphoma (MCL) PDX model and euthanized at various timepoints.
- Disease in spleen, bone marrow, and peripheral blood was assessed by flow cytometry (human CD45 and CD20 double positive cells).
- ctDNA was detectable when the spleen had less than 0.5% disease by flow cytometry.

### F. Comparing efficacy by ctDNA vs. flow cytometry after 4 weeks of dosing




- Post-treatment disease showed similar trends in both assays.
- The ctDNA assay was able to identify a significant difference between the two best responding arms which appeared to have near complete responses when assessed by flow cytometry alone.
- The fold difference between the two best responding arms was 1.1-fold by flow cytometry and 3.6-fold by ctDNA, illuminating a greater disparity in MRD and relapse potential between these 2 treatment groups.

## Summary & Conclusions

- We developed a novel assay to detect ctDNA from xenograft models using ddPCR targeting multicopy human genetic sequences.
- Disease detection by plasma ctDNA correlated with other established assays such as IHC, BLI and flow cytometry in multiple types of disseminated models.
- Work is ongoing to explore utility in solid tumor models and feasibility of incorporating mutation-specific primers.

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