

Postoperative Lymphatic Exudate as a Proximal Liquid Biopsy source in Muscle-invasive Bladder Cancer

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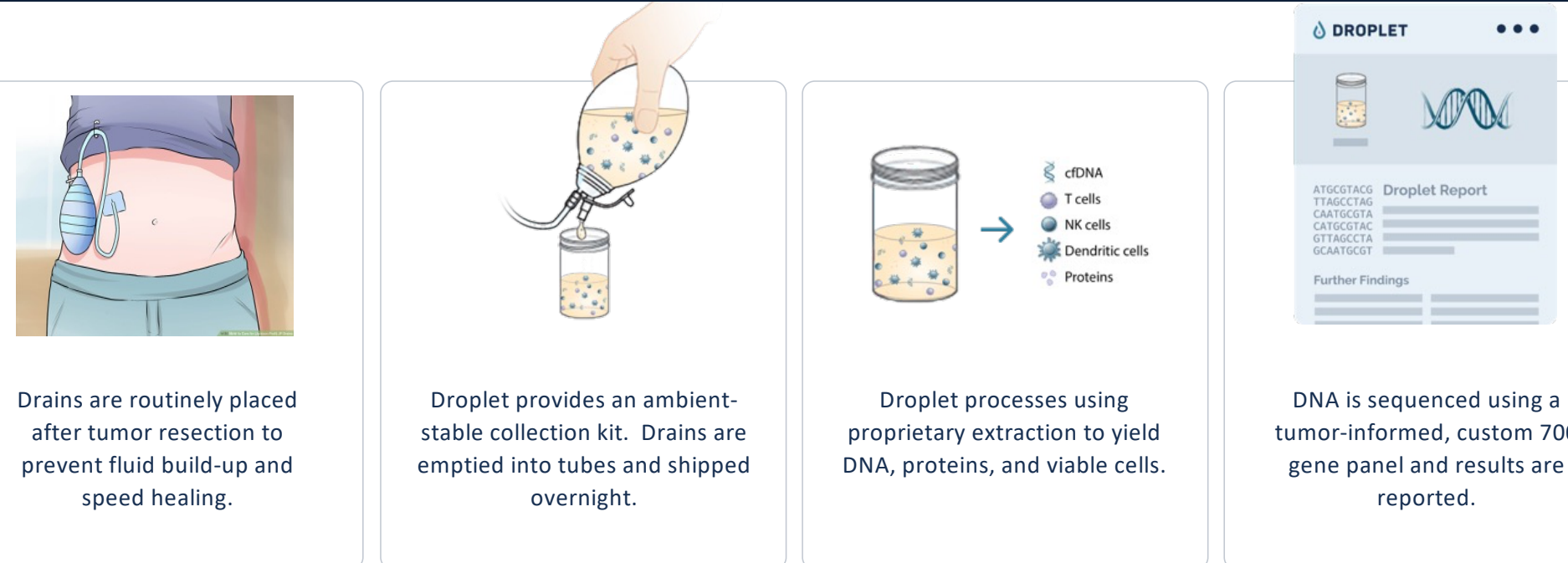
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Introduction

Patients with muscle-invasive bladder cancer (MIBC) experience recurrence rates of up to 50% following radical cystectomy^{1,2}. Current monitoring involves imaging scans or peripheral blood ctDNA^{3,4}, however, there is unmet need for a sensitive molecular test that can identify recurrence early enough to more precisely tailor adjuvant therapy. Our team has pioneered the use of lymphatic exudate collected via surgical drains ("lymph") as a proximal liquid biopsy fluid. We have previously shown that lymph collected 24 hours after surgery identified molecular residual disease (MRD) in head and neck squamous cell carcinoma (HNSCC) in the immediate post-surgical window^{5,6}. Here, we evaluate the feasibility of postoperative lymph to enable sensitive detection of MRD and characterize the dynamics of circulating tumor DNA (ctDNA) in lymph from MIBC patients for 96 hours after surgery.

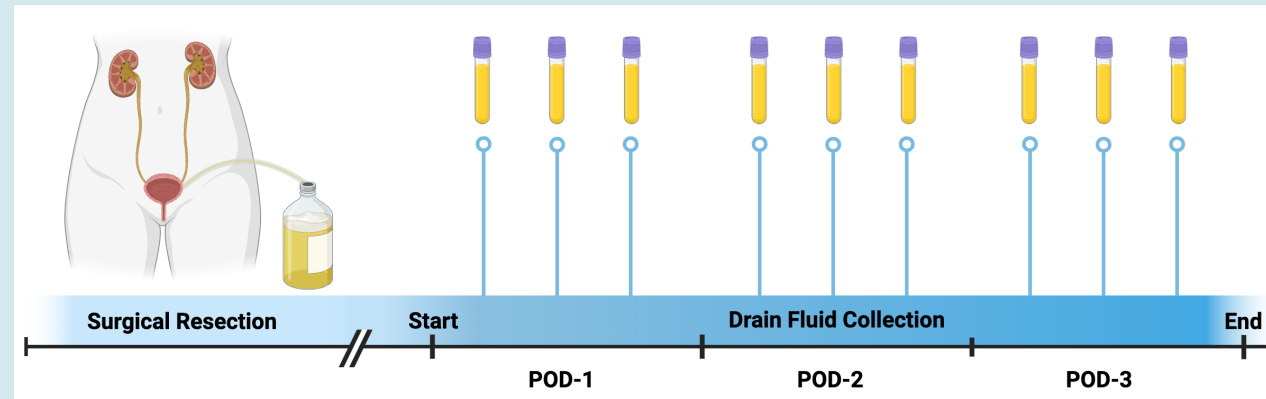
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Methods and Materials

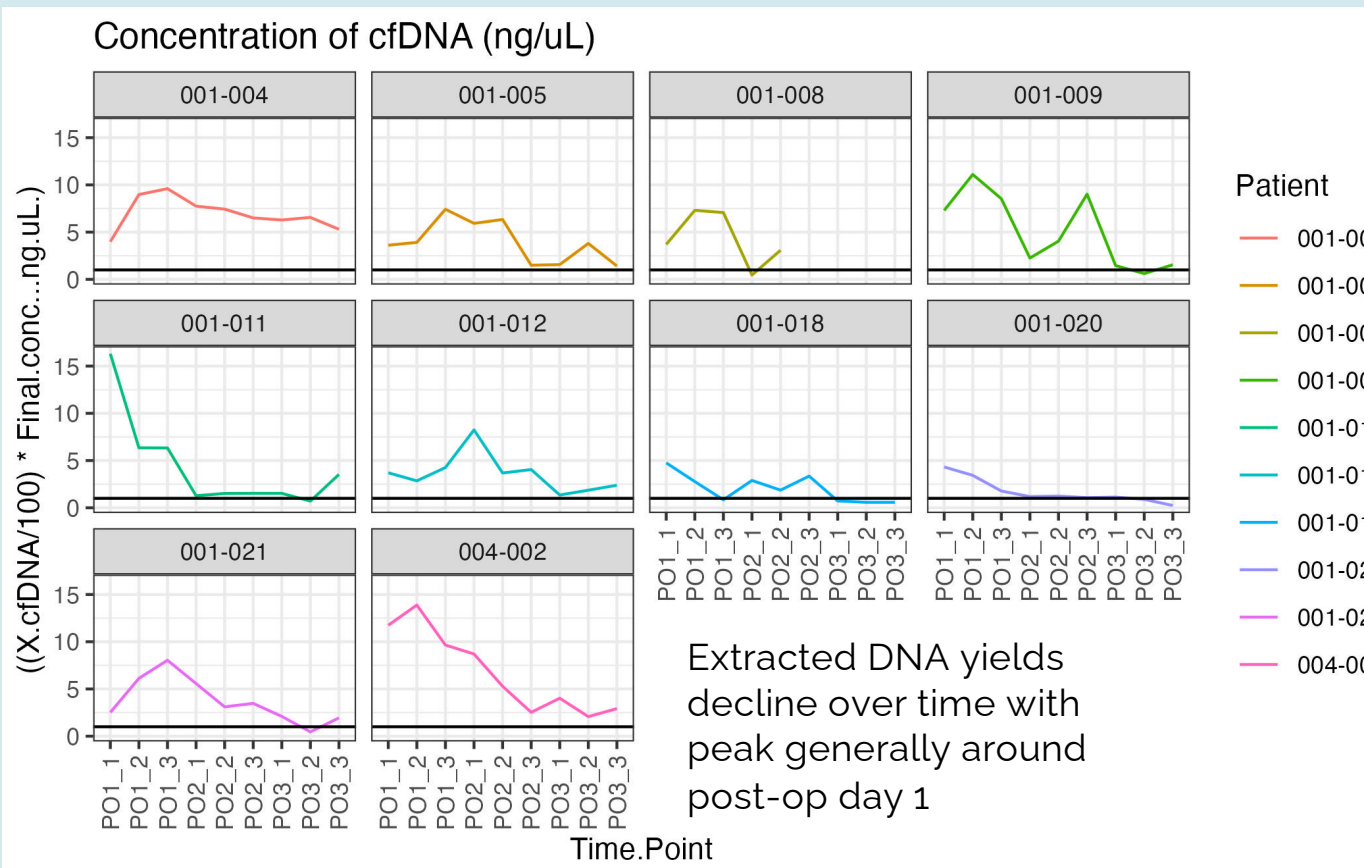
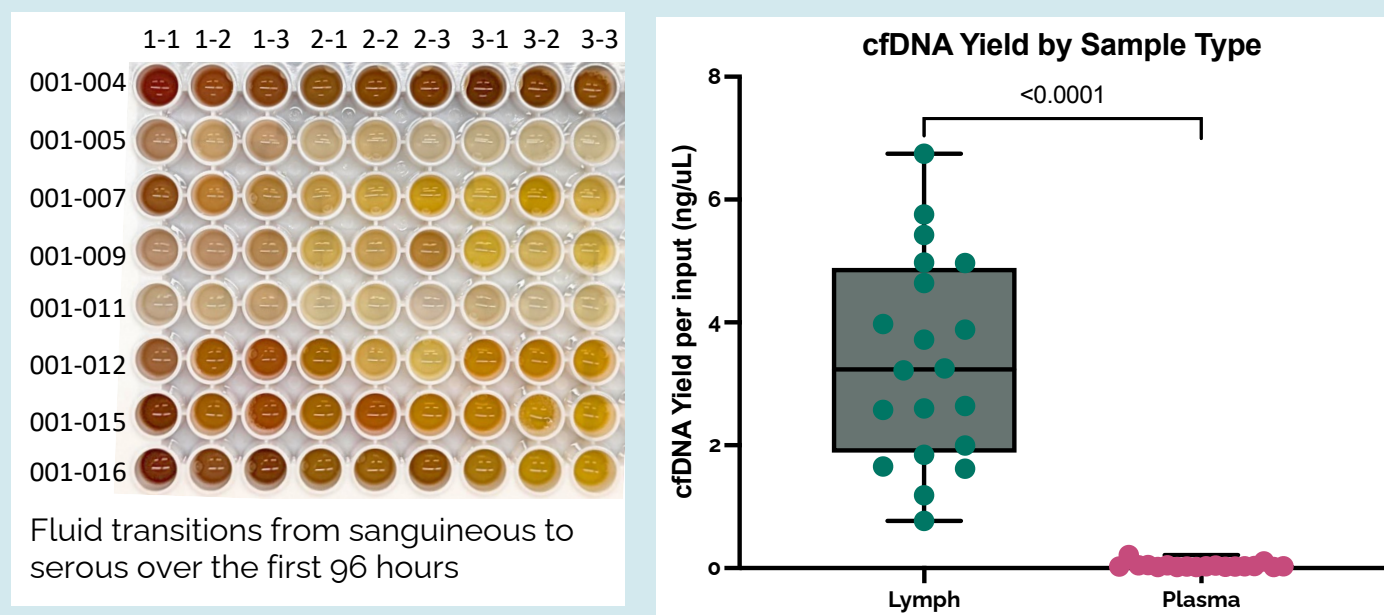


Lymph, tumor and whole blood were prospectively collected from 8 MIBC patients undergoing radical cystectomy: 4 patients with disease recurrence (REC) and 4 with no evidence of disease (NED) with >1 year of follow-up. Tumor and peripheral blood were collected at surgery; lymph at 24 ± 6 hours after surgical close. For the serial cohort (n=10), lymph was collected every 8 ± 4 hours for 96 hours after surgery. Next-generation sequencing libraries were prepared from genomic and cfDNA using a 500 gene pan-cancer hybrid capture panel; deduplicated coverage was >250X for tumor and blood and >2500X for lymph. Somatic mutations were identified in tumor with matched blood. Tumor-specific variants were directly genotyped in lymph using a custom bioinformatic pipeline. Mutation calls were filtered by a base-specific error model to eliminate artifacts.

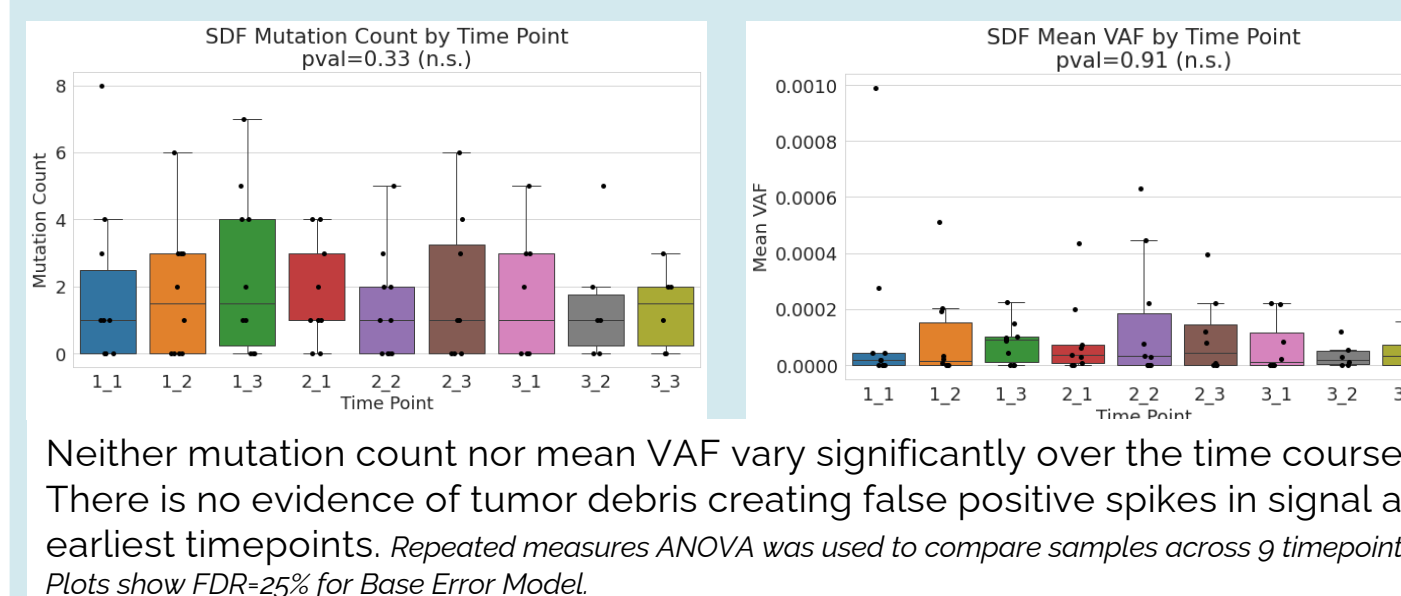
Longitudinal ctDNA dynamics in post-surgical lymph



Post-surgical lymph is a rich source of cfDNA

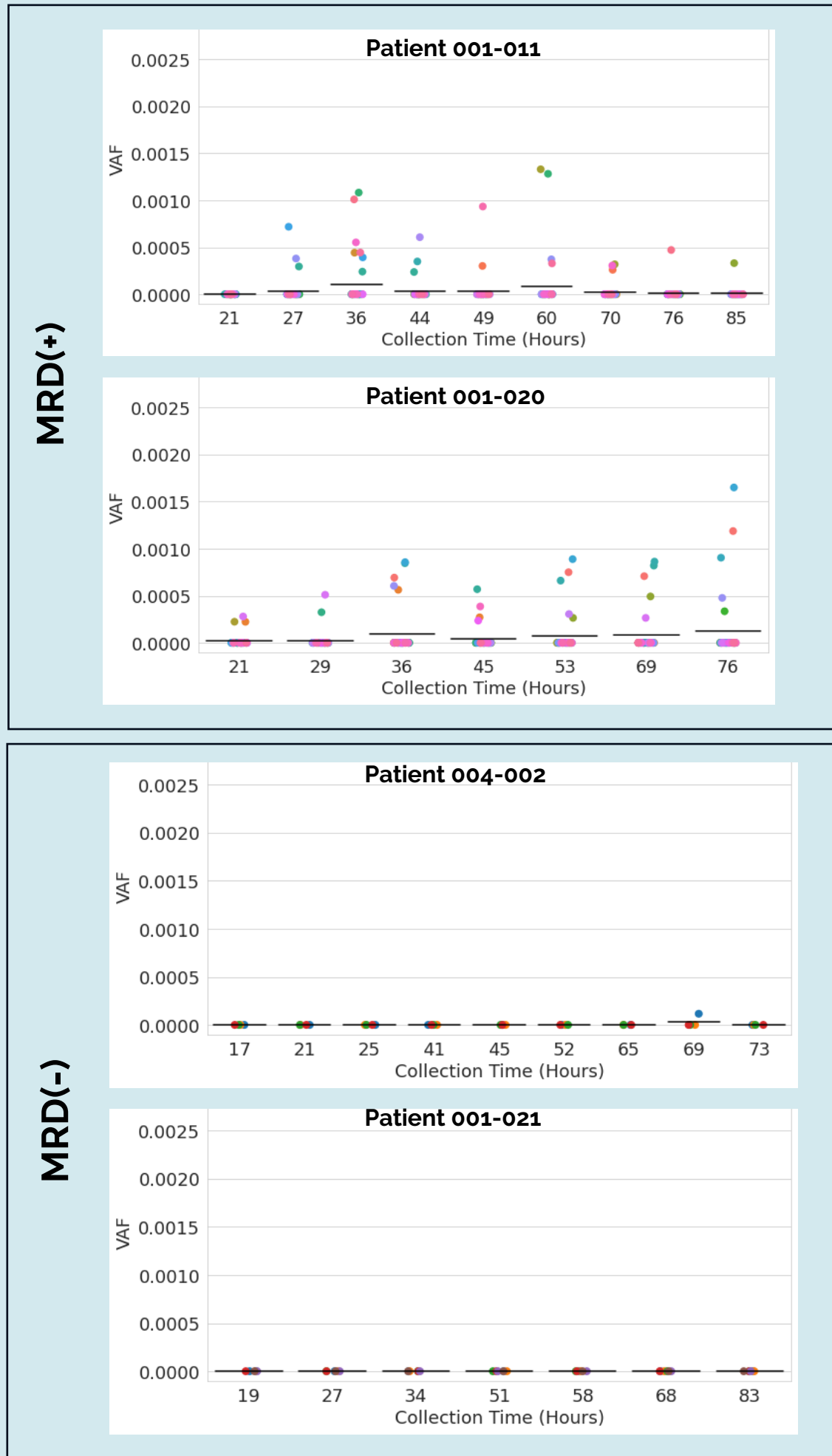


No significant difference in tumor signal over time



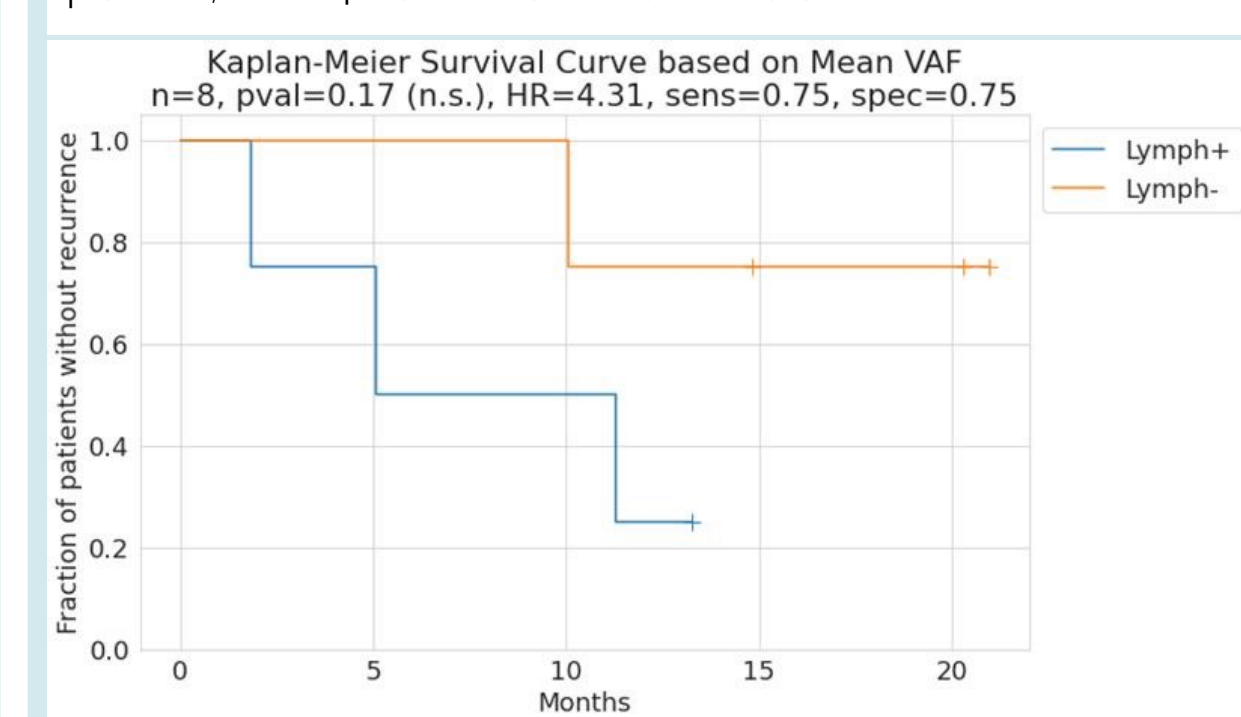
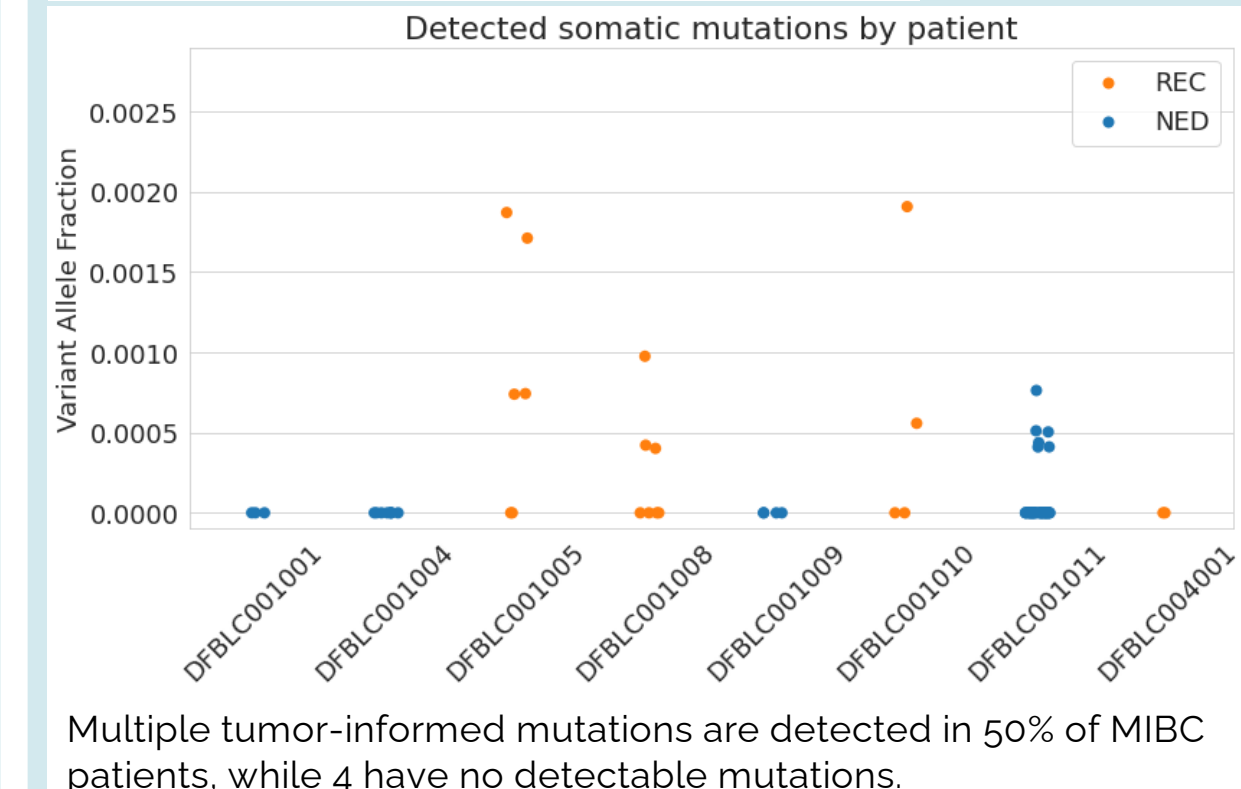
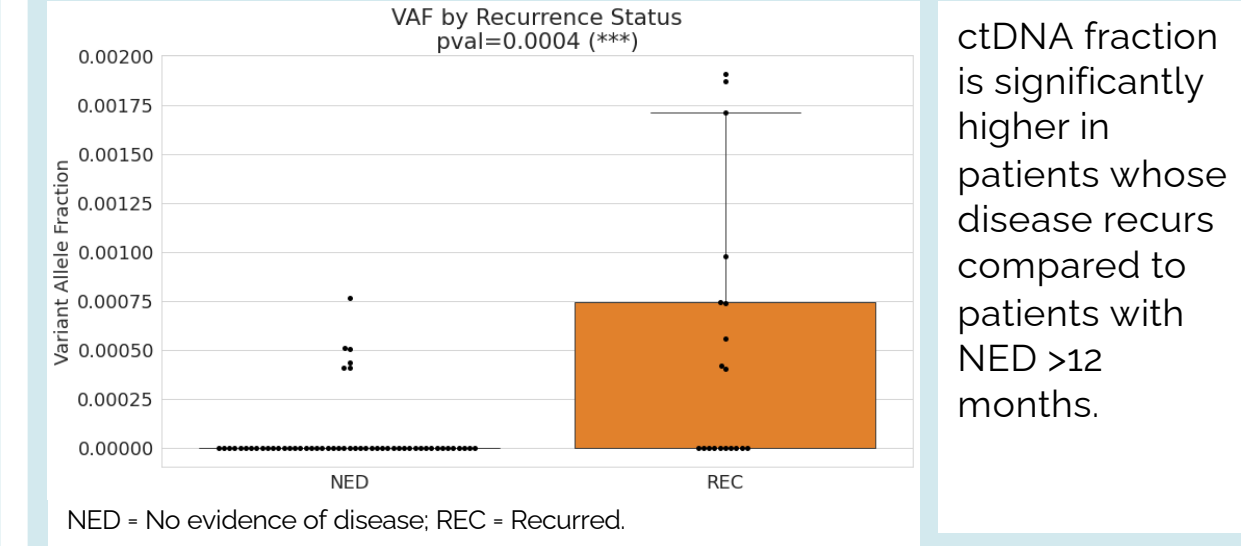
Results

MRD results are consistent across timepoints



VAFs of individual patients were consistent (both MRD+ and MRD-) across timepoints. There is no obvious benefit to collecting fluid at either earlier or later timepoints. Stochasticity showed some impact to MRD classification.

ctDNA is increased in patients who recur



While non-significant in this small cohort, the HR and sensitivity in this feasibility pilot are consistent with published larger studies in HNSCC.

Conclusions

We demonstrated the feasibility of analyzing ctDNA in post-surgical lymph in MIBC. ctDNA levels were stable across the first 96 hours after surgery, a pattern consistent with ongoing shed from residual tumor as opposed to decay of DNA from resected tumor debris. While non-significant in this small cohort, the HR and sensitivity in this feasibility pilot are consistent with published larger studies in HNSCC. If these results generalize, postoperative lymph MRD testing has the potential to provide more personalized adjuvant treatment decision-making in MIBC patients.