SCALABLE, RELIABLE RECOVERY OF INTACT DNA FROM LYMPHODEPLETED SAMPLES FOR CAR-T VECTOR ANALYSIS

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Introduction

- Chimeric antigen receptor T-cell (CAR-T) therapies are key gene therapy for both oncology and autoimmune diseases. CAR T-cells and other CAR–immune cells as well as TCR-T therapies are produced predominantly by lentiviral/retroviral vector transduction.
- Safety and pharmacokinetic monitoring of patients by molecular methods is required in CAR-T therapies[1]. Safety monitoring is done by testing for replication competent lentivirus/retrovirus (RCL/R) with qPCR and pharmacokinetics is assessed by testing for vector copy numbers (VCN) with ddPCR. RCL/R and VCN assays need genomic DNA isolated from patient's whole blood (WB) samples.
- CAR therapies, autologous or allogeneic, need patients to be lymphodepleted prior to infusion of CAR T-cells. As a result of lymphodepletion, patients have very few or no lymphocytes in blood within the first few days of CAR-T therapy.
- Lymphocytes are necessary for RCL/R and VCN monitoring at specific time points before and after CAR T-cell infusion. VCN is performed from day (d) 0 and ≥ d28 of CAR therapy at predetermined intervals. In this phase, limited number of lymphocytes (DNA) is available before recovery of the lymphocytes including the CAR T-cells in the patient.
- To ensure sufficient DNA is available for CAR monitoring during the first 2 weeks, DNA should be extracted using as much sample input volume as possible. This poses challenges to any automated extraction procedure as well as for efficiency of downstream molecular assays.

Objective

1. To isolate sufficient DNA, for VCN and RCL/R testing from patient's undergoing CAR/TCR-T cell therapies, using automated nucleic acid isolation platform.
2. To validate the nucleic acid isolation process for clinical samples

Materials and Methods

- Peripheral venous blood (WB) samples from healthy volunteers, obtained after informed consent, was used. Nucleic acid isolation (NAI), was performed by us with 2 different automated platforms.
- Concentration of isolated DNA was determined by Qubit (v4.0, ThermoScientific) fluorometer. Quality of the isolated DNA was determined by agarose gel electrophoresis.

Results

I. Improving DNA yield

- We first found that increasing the volume of WB input does not increase the yield of DNA (figure 1).
- We then tested different workflows to maximize the yield of isolated DNA.
- We identified a workflow with the automated platform that gave us better yields of DNA (figure 2).

II. Validating the automated isolation workflow

- We then validated[2] the optimized workflow with 2 different operators (Op1 and Op2) on different days with multiple samples.
- The results showed that DNA yield was scalable with volume of sample input (figure 2).
- We further verified the isolated DNA with our validated RCL and VCN assays using spike-in controls[2,3]. All samples with a spike-in control were tested positive with a CV% <20% for RCL assay (qPCR) and for ddPCR: CV <20% for >50 copies/PCR and a CV< 40% for copies <50 copies/PCR. DNA samples without spike-in controls were negative.

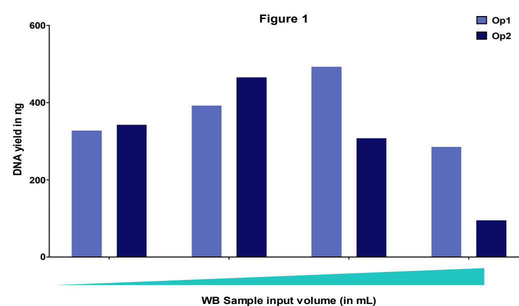

Figure 1: Verification results to determine if increasing volume of WB sample input (from 0.5mL to 4mL) improves DNA yield. Isolation was performed by 2 different operators (Op1 and Op2). Isolated DNA yield did not improve with increasing the volume of sample input. The variation between the operators was due to biological nature of samples.

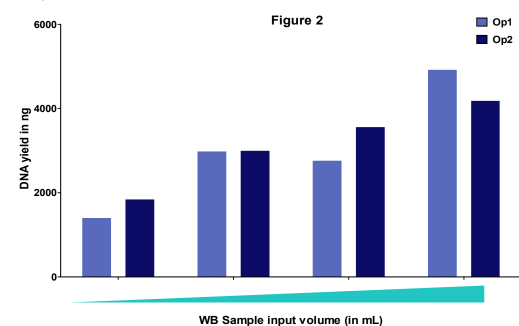

Figure 2: Validation results optimized workflow for different volumes of WB sample input (from 0.5mL to 4mL). Isolation was performed by 2 different operators (Op1 and Op2). Isolated DNA yield improved with increasing volume of sample input. Results show that DNA yield was scalable or linear with volume of sample input. The variation between the operators was due to biological nature of samples.

Conclusion

1. We have optimized and validated a scalable workflow for automated isolation of DNA from WB samples that maximizes the yield of DNA from a given sample.
2. The obtained DNA yield and quality is sufficient for safety monitoring testing (qPCR and ddPCR) in CAR-T clinical trial patient samples.
3. We are exploring the optimized workflow for total nucleic acid isolation, for both DNA and RNA.

References:
1. FDA. (2024). Considerations for development of Chimeric Antigen Receptor (CAR) T cell products - Guidance for Industry.
2. FDA. (2018). Bioanalytical Method Validation - Guidance for Industry.
3. NEN-ISO20395 (2019) Biotechnology - Requirements of evaluating the performance of quantification methods for nucleic acid target sequences - qPCR and dPCR.