Nanopore sequencing identifies transcriptomic structural variation in mouse colorectal cancer T-cell immune checkpoints: a comparison of short read (Illumina) and long read (Nanopore) sequencing technologies

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Background

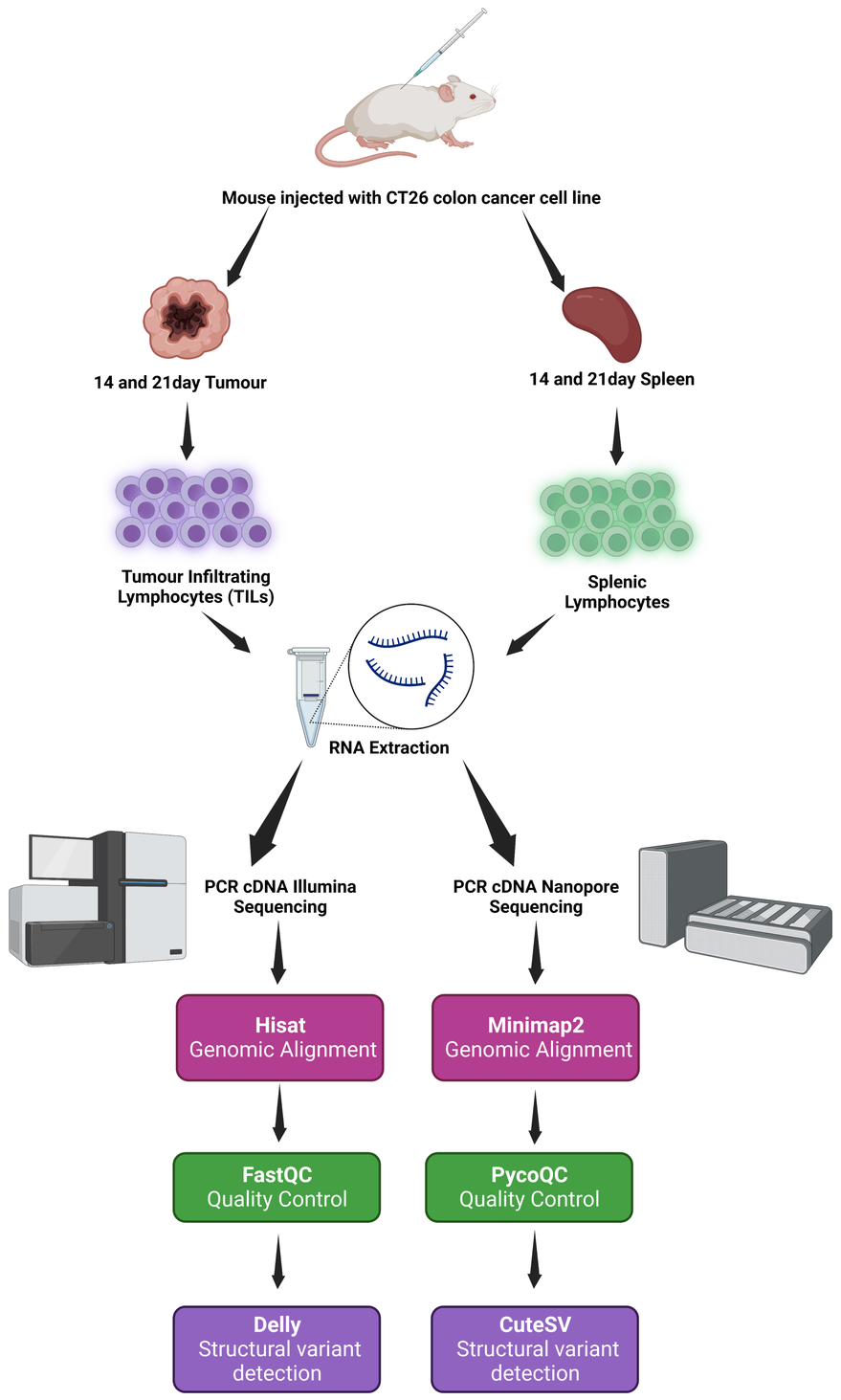
Tumour-infiltrating lymphocytes (TILs) become progressively less effective in responding to cancer. This process, called T cell exhaustion, is characterised by a temporally hierarchical loss of effector functions with increased expression of inhibitory immune checkpoints, culminating in terminally differentiated but ineffective T cell populations.

Objectives

- Identify structural variants (SVs) of inhibitory immune checkpoints in TILs at different timepoints in different T cell populations to probe mechanisms of initiation and maintenance of T cell exhaustion.
- Evaluate performance of Illumina and Oxford Nanopore technologies and associated bioinformatic tools for SV detection.

Methods

Using data from both short read (Illumina) and long read (Oxford Nanopore) sequencing, examined the transcriptomic structural variants of CD4+ and CD8+ T cells from spleen and tumour of a mouse model of colorectal cancer (CRC) at two time points (14 days and 21 days) after injection with CT26 CRC cell line.



Workflow

Results

Nanopore: better alignment and more bases mapped vs Illumina

Property	Illumina	Nanopore
Total bases (Gb)	29	22
Total reads	192,643,944	43,718,766
% Bases > Q10	99.9	97.2
% Bases > Q30	93.4	N/A
Bases mapped (Gb)	59	70
Average coverage	19.9	20.0
Mean read length (bp)	150	654
Error rate (%)	0.03	10

Nanopore: better insertion SV identification vs Illumina

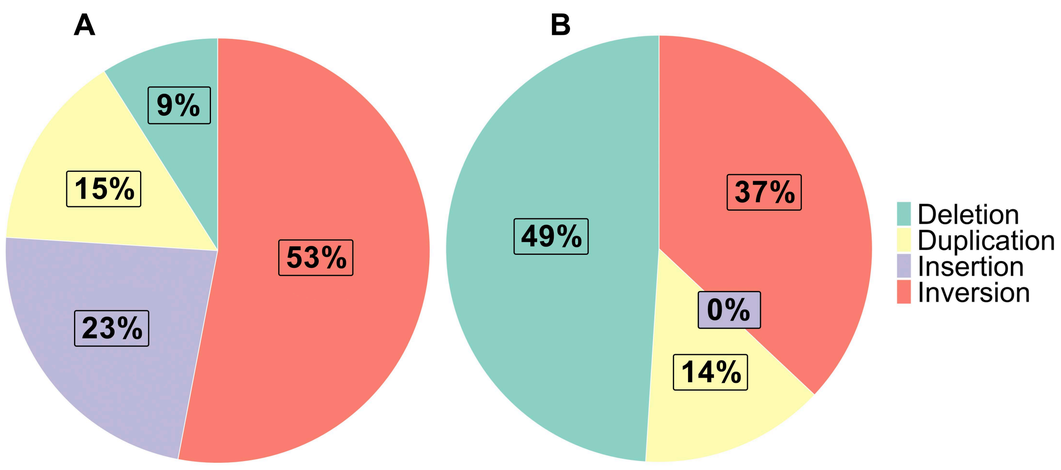


Figure 1: Percentages of SV types identified using CuteSV (Nanopore) and Delly (Illumina) among all transcripts. Percentage of SVs that are deletions (green), duplications (yellow), insertions (purple) and inversions (red) identified by (A) CuteSV with Nanopore data and (B) Delly with Illumina data.

Nanopore: novel SVs identified within clinically targeted inhibitory immune checkpoints

Gene	Location	Structural variant
Tim3	11:46346626-46346626	Insertion
Klrc1	6:129642506-129642506	Insertion
Klrc1	6:129642505-129642505	Insertion
Klrc1	6:129643759-129643759	Insertion
Pdcd1	1:93965478-93965478	Insertion
Pdcd1	1:93968034-93974124	Duplication
Pdcd1	1:93969026-93969151	Inversion
Pdcd1	1:93968969-93969159	Inversion
Pdcd1	1:93969188-93969239	Inversion
Tigit	16:43468687-43468687	Insertion
Tigit	16:43469425-43469425	Insertion

Table 2: Novel SVs identified by CuteSV within clinically targeted inhibitory immune checkpoints. CuteSV identified a wide range of previously unidentified SVs including duplications, insertions and inversions among inhibitory immune checkpoints (Tim3, Klrc1, Pdcd1 and Tigit). Insertions were the most common SV identified, followed by inversions and duplications.

The number of inhibitory immune checkpoint SVs over time

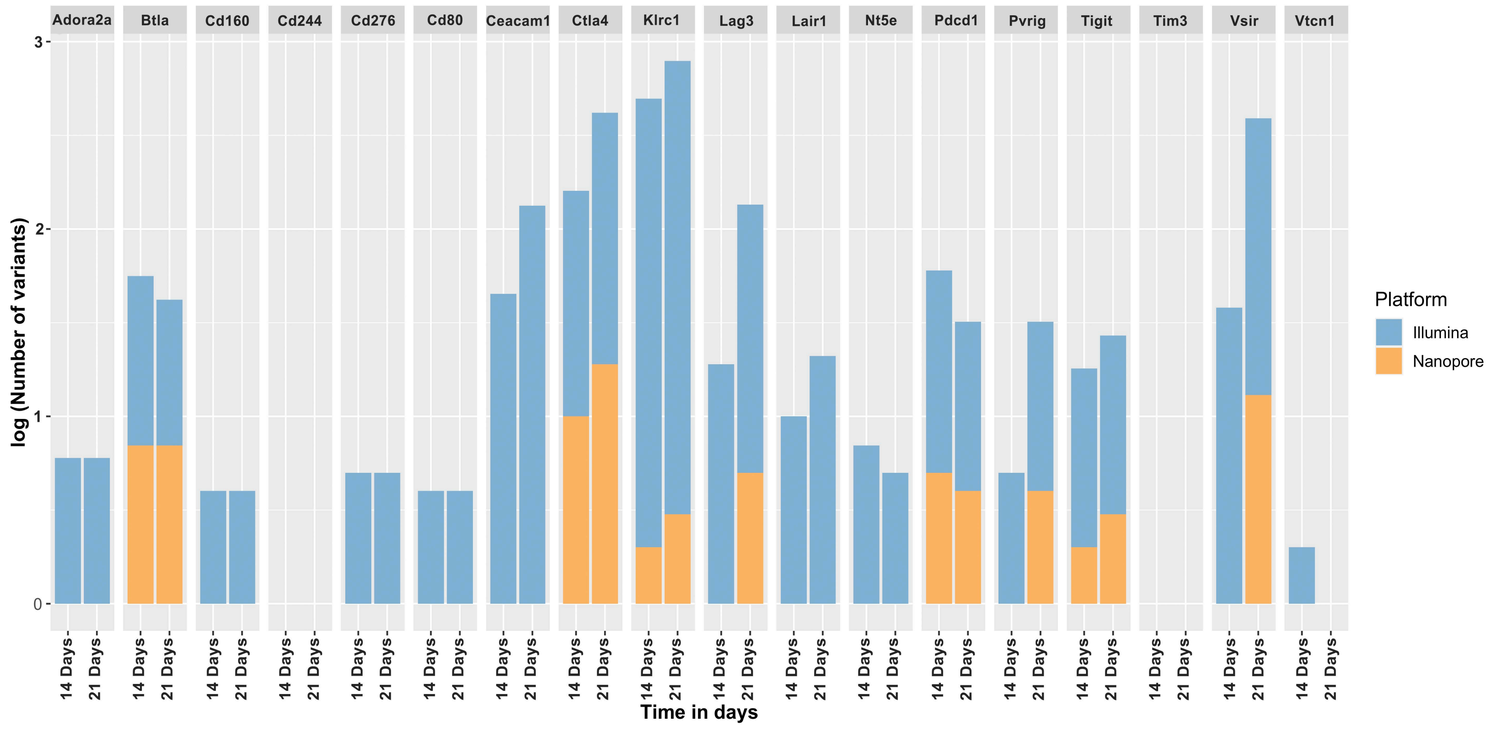


Figure 2: Inhibitory immune checkpoint SVs identified in CD4+ and CD8+ T cells harvested from spleen and tumour of a colorectal cancer mouse model 14 days and 21 days after injection with CT26 CRC cell line. Sequencing data was obtained using both Nanopore and Illumina technologies. Sequences were aligned to the reference genome mm39. Bioinformatic tools including CuteSV and Delly were used for analysis of Nanopore long read data and Illumina short read data, respectively. The results were processed with Ensembl Variant Effect Predictor.

Conclusions

- Nanopore: better alignment and higher percentage of bases mapped vs Illumina
- Nanopore: better insertion SV identification vs Illumina
- Moderate increase in SVs over time in key inhibitory immune checkpoints (Ctla4, Klrc1, Lag3, Tigit)
- Novel structural variants within clinically targeted inhibitory immune checkpoints could be identified using Nanopore data

References

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