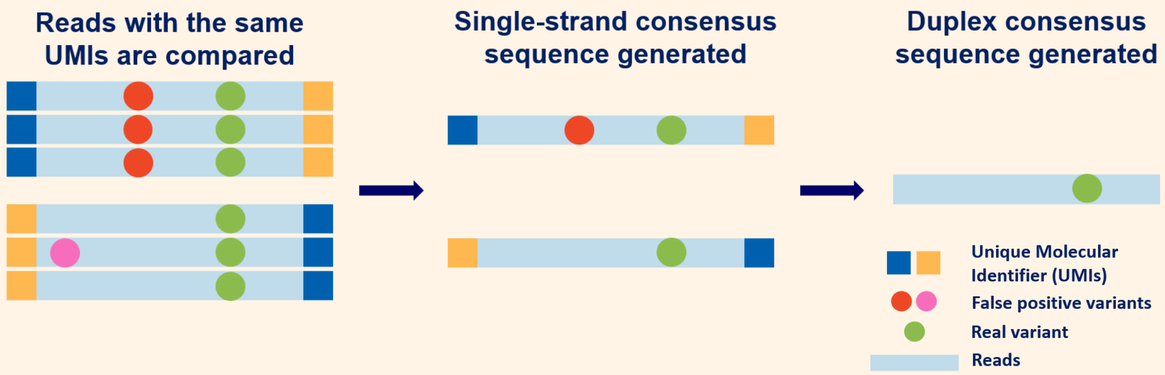
Tracking treatment resistant clones in adult Acute Lymphoblastic Leukaemia

Priscilla Ardianto (200769910), Dr. Sarra Ryan, Dr. Richard Yim, Professor Anthony Moorman, Dr. Thomas Creasey

Institute of Translational and Clinical Research, Newcastle University, Email: p.ardianto2@newcastle.ac.uk

BACKGROUND

- Acute lymphoblastic leukaemia (ALL) is characterised by malignant transformation of haematopoietic stem cells of lymphoid lineage.
- 90% survival in children (1-9 years old), but only 10-20% survival in older adult patients (≥55 years old).
- There is evidence that secondary abnormalities, such as point mutations can drive treatment resistance, leading to poor survival and relapse.
- Tracking these abnormalities through treatment using Next Generation Sequencing (NGS) can lead to better understanding of the genetic profile which can be used to improve ALL management in older adults.
- Duplex Sequencing (DS) is an NGS library analysis method developed to eliminate false positives introduced by PCR errors which entails the generation of consensus sequence

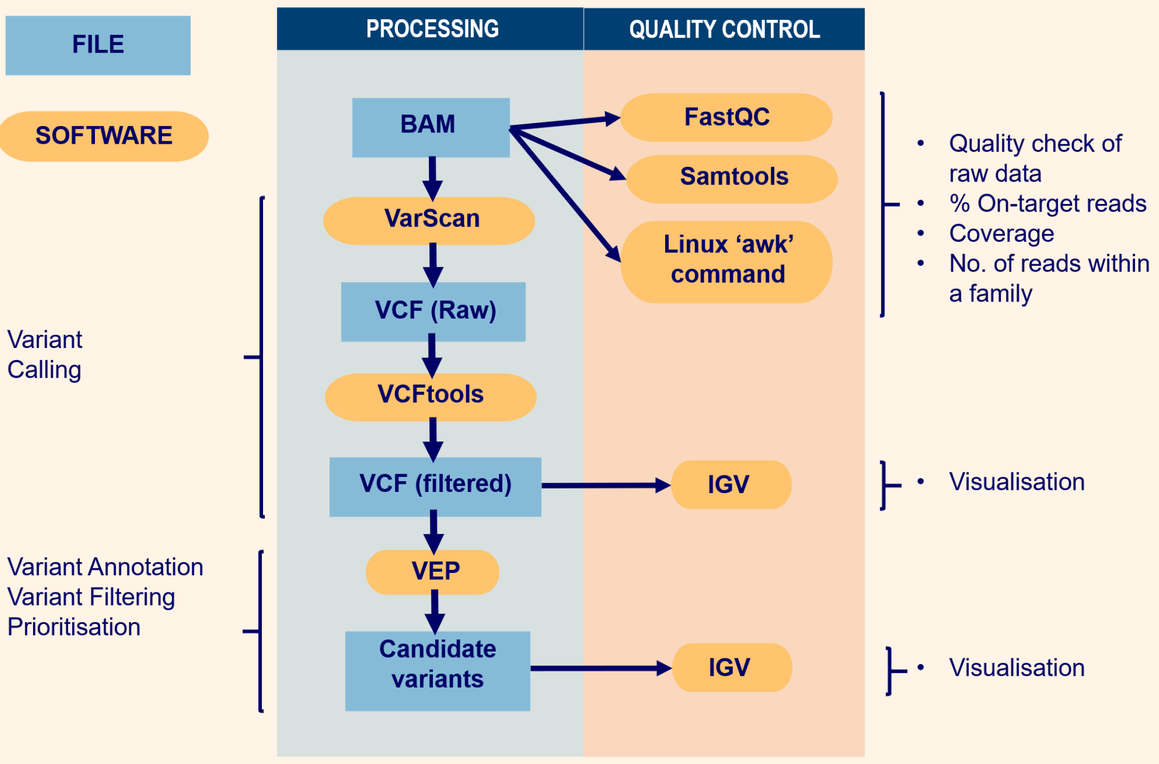


AIMS

1. To perform variant identification on Duplex Sequencing output and compare it with prior output from Whole Exome Sequencing (WES)
2. To track the rise and fall of variant levels through treatment

METHODS

Targeted sequencing incorporating Duplex Sequencing was performed on 22 serial samples collected from ALL patients aged >50 years old. The resulting BAM file output were then processed using the pipeline illustrated below to generate a list of candidate variants. The variant allele frequency (VAF) of each identified variant were then tracked through samples taken during treatment.



RESULTS

1 Comparison between DS and WES

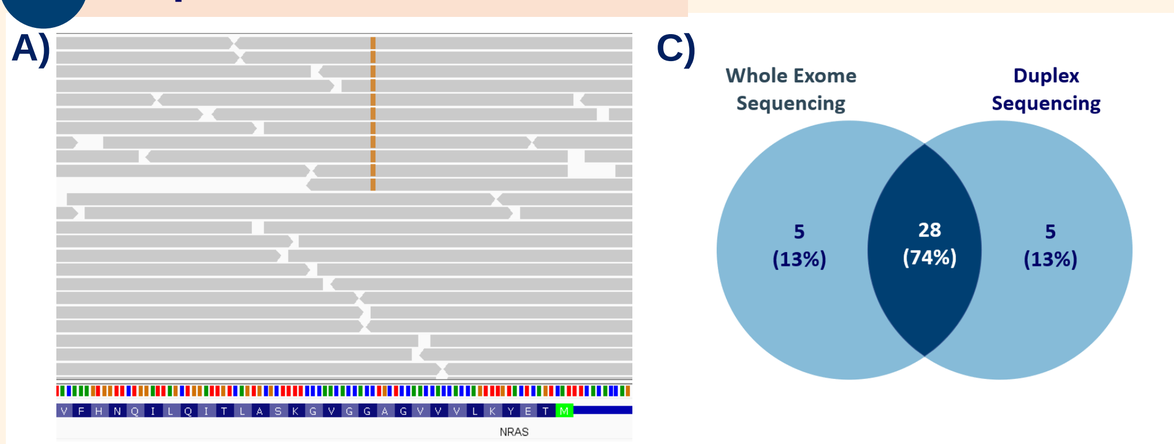


Figure 1. (A) IGV visualisation of DS output showing an *NRAS* p.G12R variant that was solely identified by DS. **(B)** IGV visualisation of the same sample and location on a WES output. The output had background variants introduced by PCR error which decreased the practical sensitivity of WES. **(C)** Concordance of calls made by DS and WES. Most of the calls solely made by WES were false positives. Calls unique to DS had VAF below the detection limit of WES.

2 Variants identified in the patient cohort

A)

PATIENT	AGE	SEX	SUBGROUP	SNVs
29779	87	M	Unknown	5
29780	55	F	B-other	6
29854	75	F	BCR-ABL1	5
30142	66	F	B-other	9
30643	52	M	IGH-CRLF2	6
31044	63	F	HoTr	3

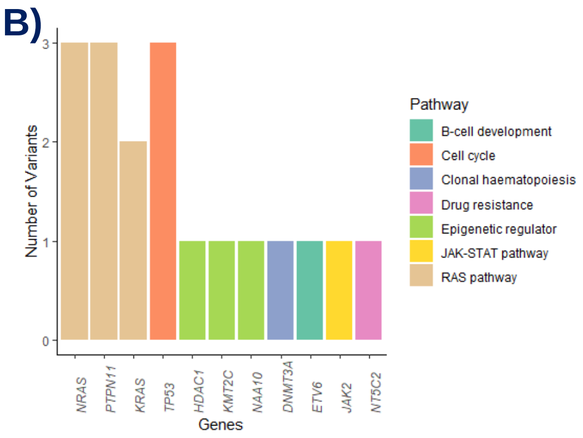


Figure 2. (A) The number of variants identified in each patient. All variants identified were SNVs. There was no correlation between the number of variants with patient's age, sex, and risk group at diagnosis. **(B)** Common pathway alterations in ALL studies that were found in the patient cohort. Alterations in the RAS pathway predominate.

3 Variant tracking patterns

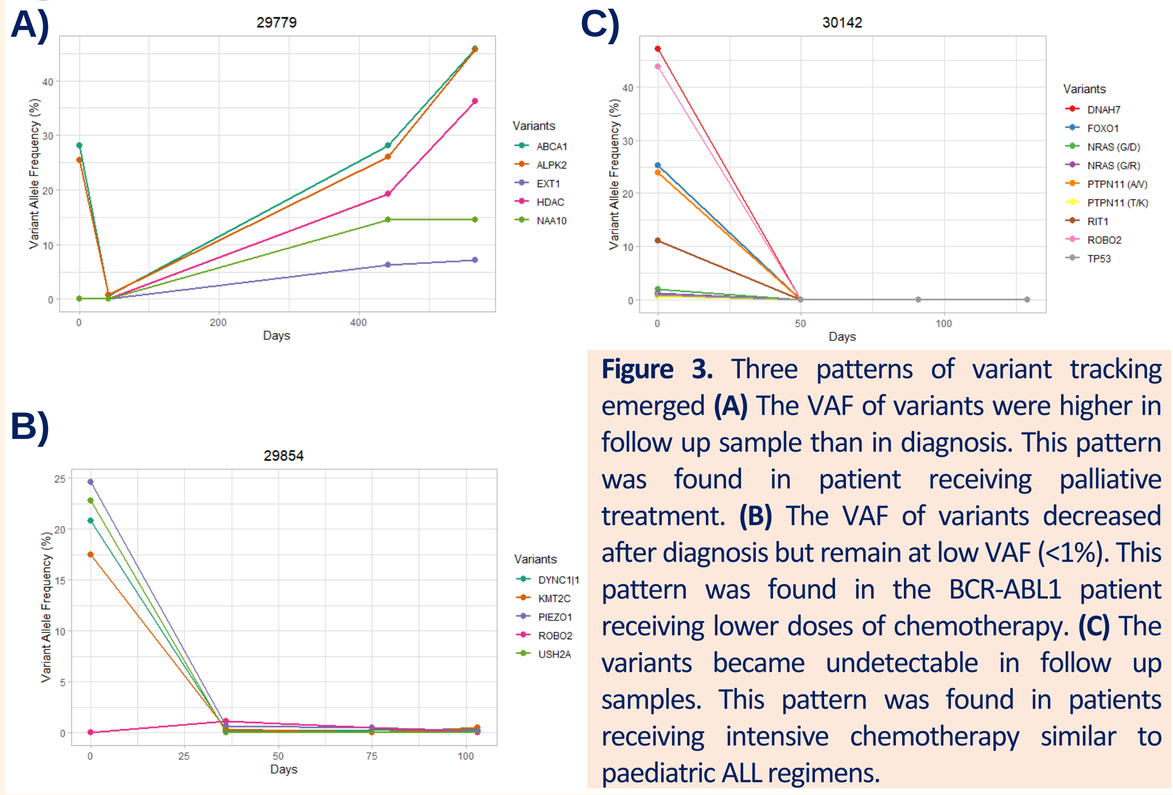


Figure 3. Three patterns of variant tracking emerged **(A)** The VAF of variants were higher in follow up sample than in diagnosis. This pattern was found in patient receiving palliative treatment. **(B)** The VAF of variants decreased after diagnosis but remain at low VAF (<1%). This pattern was found in the BCR-ABL1 patient receiving lower doses of chemotherapy. **(C)** The variants became undetectable in follow up samples. This pattern was found in patients receiving intensive chemotherapy similar to paediatric ALL regimens.

CONCLUSION

DS is more reliable than WES in detecting low level variants as it is able to eliminate false positive calls introduced by PCR errors which increases its practical sensitivity. RAS pathway mutations predominate in this patient cohort. Administering intensive chemotherapy in 1st induction phase results in the clearance of both clonal and subclonal variants. Meanwhile, variants were found to persist in patient receiving lower dose of chemotherapy. This result seems to support the use of paediatric-like intensive chemotherapy in older adult patients, especially in the initial induction phase, provided that the level of toxicity can still be tolerated.