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Sequencing of circulating tumour DNA reveals additional driver mutations in Richter's Transformation

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LAY SUMMARY

Richter's transformation and the STELLAR trial

Richter's transformation (a.k.a Richter's syndrome) is a rare complication of CLL in which the cancer cells transform into an aggressive lymphoma. The STELLAR trial examines if adding acalabrutinib to standard chemotherapy can improve the responses and outcomes for Richter's transformation.

Changes in the chromosomes and DNA (mutations) in cancer cells obtained at biopsy (including in CLL and Richter's transformation) help doctors and scientists understand how the disease behaves and can help develop treatments.

Circulating tumour DNA

Cancers release fragments of their abnormal DNA into circulation, where they can be detected in the blood plasma. In other cancers, testing of plasma circulating tumour DNA gives a picture of the DNA changes in the tumour using a blood test. Changes in the amount of circulating tumour DNA after treatment can identify early in the treatment which patients have good and poorer responses.

What we did

We sequenced the ctDNA from 35 patients with Richter's transformation enrolled in the STELLAR trial. We compared the results from the ctDNA with the results from sequencing the lymph node biopsy. Finally, we looked at how the ctDNA changed after two cycles of treatment.

What we learnt

All patients had mutations detected in their plasma before treatment. More patients had *TP53* mutations in the plasma circulating tumour DNA than in the tissue biopsy. Most patients (29/31) still had mutations detectable after two cycles of treatment.

ACKNOWLEDGEMENTS

This trial is funded by Blood Cancer UK under the Trials Acceleration Programme (TAP). Acalabrutinib is required free of charge by AstraZeneca. An unrestricted educational grant was provided to support the trial & adjunctive science by AstraZeneca. The views & opinions expressed therein are those of the authors & do not necessarily reflect those of AstraZeneca, Blood Cancer UK, the NHS, or the Department of Health.

THE STELLAR STUDY FOR RICHTER'S TRANSFORMATION

Table 1	Randomised Cohort	Platform Cohort 1	Platform Cohort 2
Inclusion Criteria	Newly diagnosed *de novo* DLBCL-type Richter Transformation Anthracycline naïve RT did not occur on BTK-inhibitor	Relapsed RT following R-CHOP	Anthracycline naïve RT developed within 4 weeks of ibrutinib
Treatment	1:1 randomisation between 6 x R-CHOP versus 6 x R-CHOP plus acalabrutinib	Acalabrutinib monotherapy	R-CHOP + Acalabrutinib
Primary Objective	PFS	ORR	
Secondary Objectives	Overall Survival, Quality of Life Treatment-related toxicities Proportion of RT patients receiving cellular therapy		
Exploratory objective	MRD by flow-cytometry (10^{-4}/L) Relationship between ctDNA parameters and clinical outcomes		
MRD measurable residual disease; ORR Overall Response rate (Complete and Partial Response); PFS Progression Free Survival;			

PLASMA CELL-FREE DNA SEQUENCING

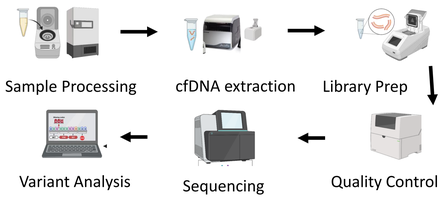


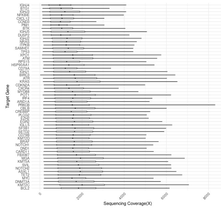
Figure 1: Summary of cell-free DNA sequencing workflow

The 56 gene capture panel targets reflect variants contributing to the pathogenesis of B-cell malignancy, reported in RT or amenable to targeted therapy. Libraries were prepared using the Thruplex-IDT Tag-Seq kit and sequenced on the Illumina NextSeq.

The ctDNA burden was calculated by multiplying the total cfDNA concentration (HGE/ml) by the median allele variant fraction.

RESULTS: CTDNA PRIOR TO TREATMENT

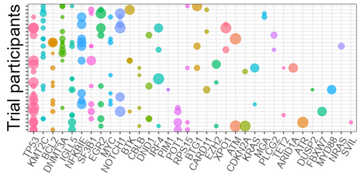
Figure 1: Cell-free DNA sequencing depth of coverage



Legend 1: Coverage by target gene

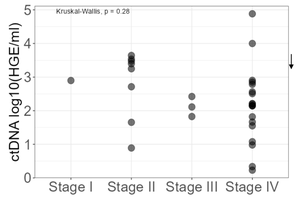
Table 2: STELLAR Participants (n=37)	
Gender	23 m; 14 f
Age (Median, Range)	72 years(39-84)
Stage I	1 patient
Stage II	8 patients
Stage III	4 patients
Stage IV	10 patients
Data missing	4 patients
Known aberrant *TP53*	6/37 (16.2%)
B-symptoms	13/37 (31.2%)
Bulky disease	14/37 (37.5%)

Figure 3A: Variants in ctDNA per patient (n=35)



Legend 3A: Variants in pretreatment samples by gene (x.axis) ordered by frequency and by patient (y.axis). The size of the circle corresponds to the VAF.
170 ctDNA variants were found. Variants were detectable in all pretreatment RT ctDNA samples at median AF 0.05 (IQR 0.02-0.19). *Trial IDs not shown to comply with regulations for an ongoing trial*

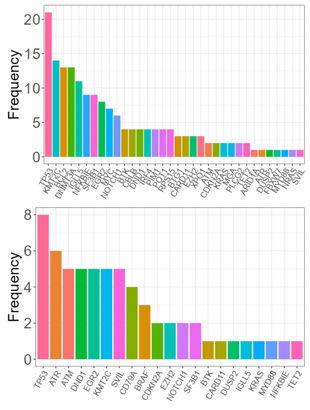
Figure 3B: Stage and ctDNA burden



Legend 3B: Pretreatment ctDNA burden log10(HGE/ml) and radiological stage. No staging data provided for 10 participants. Patients with B-symptoms had higher pre-treatment ctDNA (Wilcoxon, p =0.007).

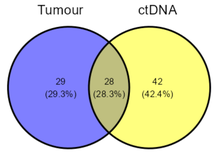
RESULTS: ADDITIONAL VARIANTS IN CTDNA COMPARED WITH TUMOUR

Figure 2A: Variants by frequency



Legend 2A: Frequency bar plot of variants detected in pretreatment ctDNA samples (top) (n=35). 21 *TP53* ctDNA variants affecting 18 patients were found. Three patients had > 1 *TP53* ctDNA variant. Eight patients had *TP53* variants found by sequencing the tumour DNA (bottom).

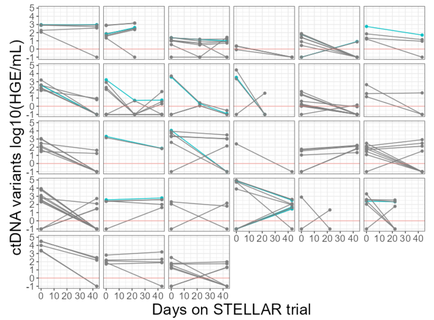
Figure 2B: Shared variants in paired samples



Legend 2B: Venn diagram showing mutations detected in the tumour only (blue, n= 29), shared mutations (overlap, n=28) and mutations found in the ctDNA alone (yellow, n= 42) for 13 RT patients with paired tumour and cell-free DNA samples sequenced.

RESULTS: EARLY DYNAMIC CHANGES IN CTDNA ON TREATMENT

Figure 4: Early changes in ctDNA variants per patient



Legend 4: Changes in ctDNA variants by log10 human genome equivalents/ml of plasma (HGE/ml) (y.axis) by time on treatment (x.axis) for patients enrolled in the randomised cohort (n=31) who submitted sequential plasma samples. The orange horizontal line refers to no ctDNA detectable and 2/31 participants had no detectable ctDNA after two treatment cycles. *TP53* variants coloured in blue. *Individual level Trial IDs not shown as before.*

CONCLUSIONS

In Richter's transformation, sequencing of plasma ctDNA detects additional variants compared to examining the tumour biopsy.

In contrast to other high-grade lymphomas studies, no clear link between ctDNA burden and clinical stage was observed, concordance between tumour and ctDNA results was limited and eradication of ctDNA after treatment was rare. Response and progression data from the STELLAR trial will enable further study of the relationship between ctDNA parameters and clinical outcomes.

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