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ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

A Personalized Whole Genome-Informed Assay Targeting Single Mutant in Circulating Tumor DNA Can Identify MRD and Predict Relapse in DLBCL

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Introduction: Minimal residual disease (MRD) detected by circulating tumor DNA (ctDNA) has emerged as a promising biomarker in diffuse large B-cell lymphoma (DLBCL). MAESTRO (minor-allele-enriched sequencing through recognition oligonucleotides) was recently developed to aid the detection of low-frequency mutations by enriching for mutant alleles using probes preferentially capturing single-nucleotide variants. We have extended this application - termed MAESTRO-Pool - to analyze personalized MRD variant detection within a cohort-level single assay. We demonstrate high sensitivity to detect MRD using MAESTRO-Pool and the detection of emergent mutations using targeted sequencing of the same samples.
Methods: Fifty-nine plasma specimens from 9 patients with relapsed/refractory (R/R) DLBCL treated on a phase II trial (NCT02362997) of post-autologous stem cell transplant (ASCT) pembrolizumab maintenance were tested. Cases were selected based on the availability of genomic DNA from tumor tissue, patient-matched germline DNA, and serial post-ASCT plasma samples (≥ 3 time points). MAESTRO probes were designed to target patient tumor-specific somatic variants using results of baseline tumor-normal whole genome sequencing. Probes were then pooled into an integrated, cohort-level assay (MAESTRO-Pool). Serial samples were compared with an orthogonal, whole-genome, tumor-informed MRD test which does not use mutation enrichment (MRD Tracker; Parsons, HA et al. *Clin Cancer Res* **26**, 2556-2564 (2020)) for sensitivity and specificity of variant detection. In addition, sensitivity to detect MRD and predict relapse was compared to that observed with immunoglobulin locus high-throughput sequencing (IgHTS). Additional baits were designed to capture single nucleotide variants (SNVs) previously reported in R/R DLBCL (63 loci in 12 genes), enabling detection of treatment-emergent mutations not identified in baseline tumor specimens.
Results: Tumor-normal WGS revealed a median of 433 somatic SNVs per tumor (range 81-1653). The pooled assay comprised 6044 SNV-specific probes. MRD identification was similar for MAESTRO-Pool and MRD Tracker. Among 59 samples, a discrepant MRD call was observed for a single sample where ctDNA was detected at 4 ppm using MRD Tracker, but not detected using MAESTRO-Pool (limit of detection [LoD] 16 ppm). Estimated tumor fractions using MRD Tracker and MAESTRO-Pool were concordant. Even with reduced sequencing requirements of MAESTRO-Pool, we observed a similar median limit of detection (LoD) for MAESTRO-Pool (median 30 ppm, range 1-18,243) and MRD Tracker (median 40 ppm, range 1-4,727 ppm).
MAESTRO-Pool identified ctDNA prior to recurrence for all 5 patients who relapsed, including at the earliest available post-ASCT timepoint for 4 of 5 relapsing patients. The time from ctDNA detection to clinical relapse (lead time) was the same or

longer for each patient using MAESTRO-Pool (median 178 days, range 69-518) compared to IgHTS (median 44 days, range not detected to 518 days) (p=0.37) (Fig.1a). MAESTRO-POOL was associated with improved sensitivity compared to IgHTS (MAESTRO-Pool sensitivity of 90.5% for samples with matched IgHTS results versus IgHTS sensitivity of 61.9%, p=0.006). Superior sensitivity was primarily driven by MAESTRO-Pool's improved detection of low-frequency (<1000 ppm) mutant alleles. In addition to tracking molecular tumor burden, we identified several de novo mutations in relapsing patients using targeted sequencing of the same samples. Notably, plasma from a patient who progressed at 18.5 months post-ASCT (DL-015) manifested an emergent CREBBP R1446H mutation not detected in the baseline tumor whose allele frequency steadily increased from 0.048% one week after ASCT to 30.533% at relapse (Fig 1b).

Conclusion: In this pilot study, MAESTRO-Pool enabled ultrasensitive detection and quantification of MRD with superior sensitivity compared to IgHTS. Complementary targeted sequencing also characterized genetic evolution, including detection of treatment-emergent mutations. Our results support the incorporation of ctDNA testing using MAESTRO-Pool in future prospective trials in DLBCL.

Disclosures Merryman: *Genentech/Roche:* Research Funding; *BMS:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Alphasights:* Membership on an entity's Board of Directors or advisory committees; *Epizyme:* Membership on an entity's Board of Directors or advisory committees; *Genmab:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Intellia:* Membership on an entity's Board of Directors or advisory committees; *Seattle Genetics:* Membership on an entity's Board of Directors or advisory committees; *Abbvie:* Membership on an entity's Board of Directors or advisory committees; *Merck:* Research Funding; *Adaptive Biotechnology:* Membership on an entity's Board of Directors or advisory committees. **Nieto:** *Affimed:* Research Funding; *Secura Bio:* Research Funding; *Astra Zeneca:* Research Funding. **Herrera:** *Adicet Bio:* Consultancy; *Caribou Biosciences:* Consultancy; *Takeda:* Consultancy; *Tubulis GmbH:* Consultancy; *Pfizer:* Consultancy; *Merck:* Consultancy, Research Funding; *Genmab:* Consultancy; *Allogene Therapeutics:* Consultancy; *Seattle Genetics:* Consultancy, Research Funding; *ADC Therapeutics:* Consultancy, Research Funding; *AbbVie:* Consultancy; *BMS:* Consultancy, Other: Travel/Accommodations/Expenses, Research Funding; *Regeneron:* Consultancy; *Karyopharm Therapeutics:* Consultancy; *AstraZeneca/MedImmune:* Consultancy; *Kite, a Gilead Company:* Research Funding; *Genentech/Roche:* Consultancy, Research Funding; *Gilead Sciences:* Research Funding; *AstraZeneca:* Research Funding. **Armand:** *MSD:* Consultancy, Research Funding; *Affimed Therapeutics:* Research Funding; *Enterome:* Consultancy; *Xencor:* Consultancy; *Regeneron:* Consultancy; *Tessa Therapeutics:* Consultancy; *Kite - a Gilead company:* Research Funding; *ATB Therapeutics:* Consultancy; *Genentech/Roche:* Consultancy, Research Funding; *Foresight Diagnostics:* Consultancy; *ADC Therapeutics:* Consultancy; *GenMab:* Consultancy; *AstraZeneca:* Consultancy, Research Funding; *IGM:* Research Funding; *Adaptive Biotechnologies:* Research Funding; *Bristol Myers Squibb:* Consultancy, Research Funding; *Merck:* Consultancy, Honoraria, Research Funding. **Murakami:** *Novartis AG:* Membership on an entity's Board of Directors or advisory committees; *imCORE (Genentech/Roche):* Research Funding. **Adalsteinsson:** *Exact Sciences:* Current equity holder in private company.

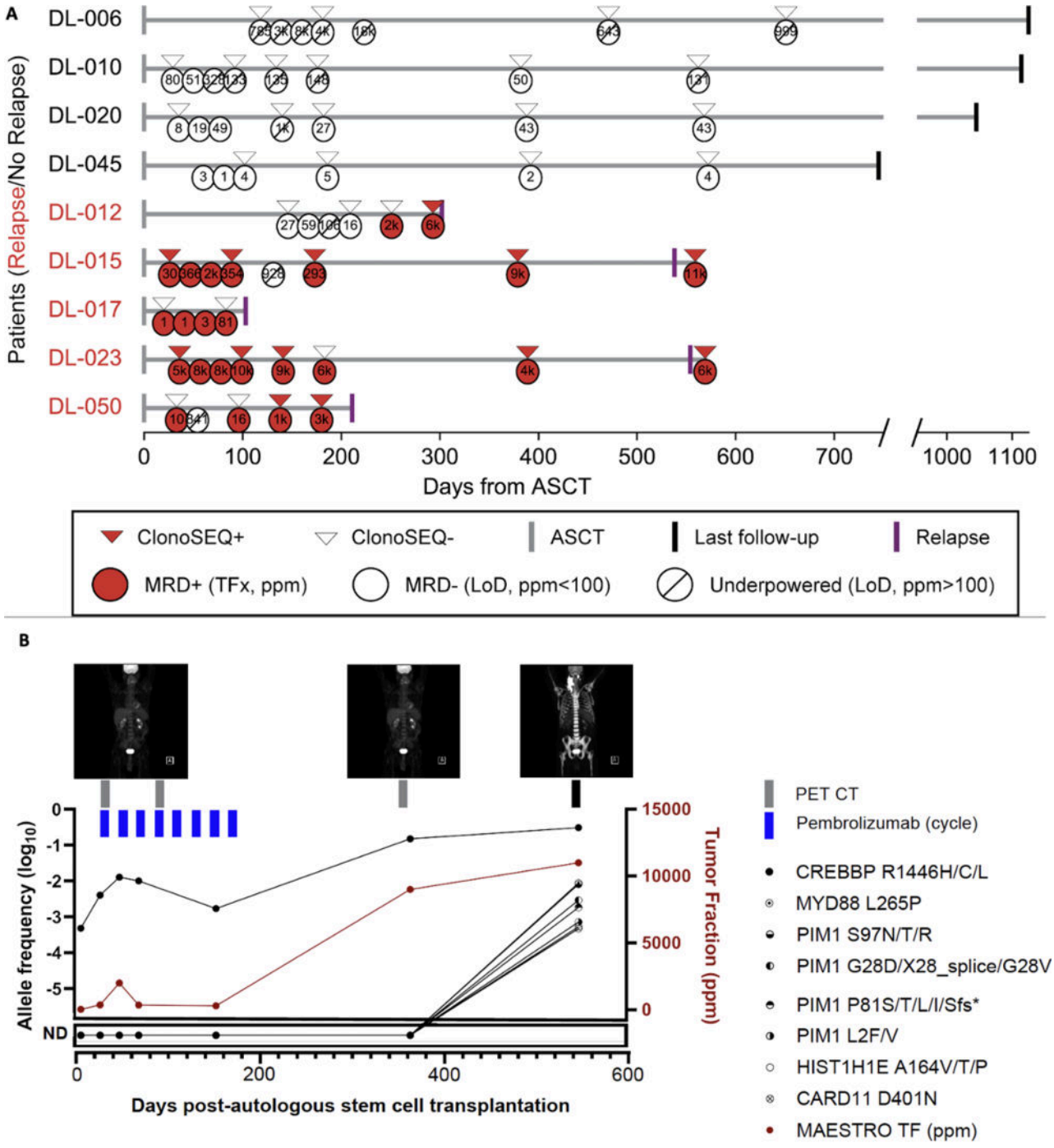

Fig 1A. Swimmer's plot showing MAESTRO-POOL MRD results along with matched ClonoSEQ results when available. **Fig 1B.** Patient vignette of tumor response dynamics shows the emergence of *de novo* mutations not present at baseline by MRD Tracker.

Figure 1

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