A NOVEL RT-ddPCR ASSAY ENABLES SENSITIVE AND ACCURATE QUANTIFICATION OF MET EXON 14 SKIPPING IN LUNG ADENOCARCINOMAS

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SUMMARY
• MET experiences exon 14 loss (METex14) in 3-4% of lung adenocarcinomas, thereby promoting protein stabilization and oncogenic pathway activation.
• Exon 14 loss or elevated MET levels are clinically actionable events susceptible to kinase inhibitors, including crizotinib and caboctibozan.
• Droplet Digital™ PCR (ddPCR™) can provide accurate copy number information of both wildtype and METex14 status using a rapid and accessible workflow.
• We describe a prototype multiplexed ddPCR assay that quantifies an internal reference gene and the fraction of METex14 skipping.

INTRODUCTION
The proto-oncogene MET experiences donor/acceptor splice site mutations that can give rise to transcripts lacking exon 14 (METex14). Although this “skipping” event occurs at frequencies comparable to ALK rearrangements in lung adenocarcinomas, current METex14 detection technologies are underdeveloped and burdened by a combination of high costs, low resolution, and/or complex workflows. Herein we report the development of a novel, accurate, and accessible 2-channel Droplet Digital PCR (ddPCR™) assay that can quantify the fraction of skipped METex14 transcripts in patient samples. Successful identification of this event correlates with MET inhibitor responsiveness, potentially benefiting treatment decisions and outcomes.

MATERIALS AND METHODS
A METex14 RT-ddPCR assay was developed using optimized reverse transcription and PCR reagents, with an optional pre-amplification step incorporated for samples with low nucleic acid quantity or quality. Gene-specific primers and junction-spanning hydrolysis probes were designed for METex14 skipping, HEK (HEK; HEX labeled), and 13/15 (METex14, FAM labeled), respectively. Droplets were generated and run on the QX200™ ddPCR platform (Bio-Rad) with a 4-μm output. Samples consisted of cell lines and a set of 125 lung adenocarcinoma FFPEs (MD Anderson) that were sequenced in parallel and generated and run on the QX200

RESULTS
MET exon 14 skipping status was determined with 100% accuracy in representative cell lines from as little as 2 ng RNA inputs. Known copy number titrations demonstrated analytical sensitivity down to 1% METex14 skipped/WT levels, exceeding the sensitivity typically achieved by other approaches. METex14 positive and negative samples were correctly identified from previously characterized FFPE specimens, including two samples that showed 27% and 95% METex14 skipping ratios, respectively.

CONCLUSIONS
• The prevalence and druggability of METex14 skipping in NSCLC makes this lesion a high-priority diagnostic target for precision medicine.
• We describe a prototype ddPCR assay that can accurately quantify the fraction of METex14 within a single multiplexed reaction.
• Further inclusion of a stable reference gene enables relative quantification of wildtype MET transcript expression independent of mechanism, and can be accommodated by both ddPCR and qPCR formats.
• The assay accepts challenging clinical specimen inputs (e.g. FFPEs) and confirms the prevalence and druggability of METex14 skipping.

References

*Research Use Only – Not for Use in Diagnostic Procedures

**Prospective research data. The performance characteristics of this assay have not yet been established.

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