Exploratory Comprehensive Epigenomic Profiling of Plasma for Non-invasive Detection of MET Activation Uncovers MET-associated Biology in Patients with EGFR-mutated Advanced NSCLC and Progression on Osimertinib

Jonathan W. Riess,[1,*] Khoi Nguyen[2,*], Sunny Das,[2] Humphrey Gardner,[2] Mike Zhong,[2] Vy Tran,[2] Tyrone Tamakloe,[2] Charlene O'Brien,[2] Hat Sawaengsri,[2] Kristian Cibulskis,[2] Aparna Gorthi,[2] Corrie Painter,[2] Matthew Eaton,[2] Ryan Hartmaier,[3] Carl Barrett[2] |
[1]UC Davis Comprehensive Cancer Center, Sacramento, CA, USA; [2]Precede Biosciences, Boston, MA, USA; [3]Translational Medicine, Oncology R&D, AstraZeneca, Boston, MA, USA. *These authors contributed equally.

BACKGROUND

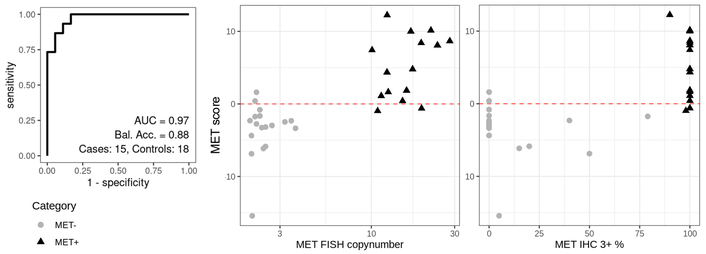
- Genomic overexpression or amplification of MET is an established bypass resistance mechanism in EGFR-mutated (EGFRm) NSCLC, observed in up to 34% of patients progressing on the EGFR tyrosine kinase inhibitor (TKI) Osimertinib.[1]
- Savolitinib, an oral, highly selective MET TKI, demonstrates clinical activity in tumors classified as MET-high by tissue-based IHC or FISH. However, tissue at progression is often inaccessible or insufficient for repeated assessment, constraining dynamic characterization of MET pathway dependence and emerging resistance mechanisms.
- To overcome these limitations, we applied a comprehensive epigenomic liquid biopsy platform to a subset of patients enrolled in the Phase II SAVANNAH trial that combined osimertinib and savolitinib after progression on osimertinib (NCT03778229), where we evaluated the feasibility of resolving MET expression from plasma, assessed its concordance with tissue-based approaches, and identified potential plasma derived therapeutically actionable markers beyond MET amplifications.

METHODS

- Baseline plasma samples from 40 patients with NSCLC, enrolled in the SAVANNAH trial following progression on first-line osimertinib, were profiled using a comprehensive epigenomic assay (Precede Biosciences, Boston, MA) from 1mL of plasma, along with downstream analyses to infer genome-wide transcriptional activity from cell-free DNA in plasma samples (Precede Bio Insight™).
- Tissue-based analysis of matched post-progression biopsies scored 15/40 tumors as MET-high/+ (FISH 10+ and IHC 3+ ≥90%) and 25 samples as MET-low/- (FISH <10, IHC 3+ <90%).
- A plasma-based MET classifier integrating inferred gene expression models and other epigenomic features, with adjustment for estimated ctDNA fraction effects, was applied to resolve MET status. Performance was evaluated by cross-validation and assessed for concordance with tissue-based classification.
- Plasma samples were also assessed for potential resistance markers, using inferred gene expression models for select genes, and a previously described test for SCLC histology.[2] In parallel, pathway analyses on genome-wide differential plasma epigenomic activity was used to define MET-associated biology.

RESULTS

Figure 1: Precede's Plasma MET Test Accurately Predicts Tissue-based MET Status



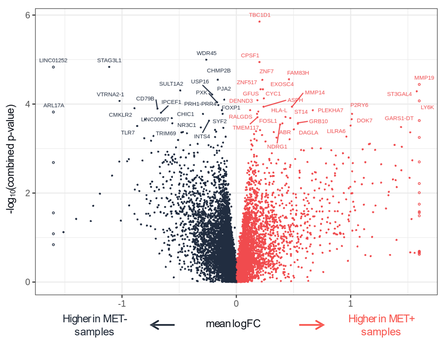
Performance of the Precede plasma-based MET test in predicting tissue-based MET status.
Left panel: Receiver operating characteristic (ROC) curve demonstrating classification performance (AUC=0.97; balanced accuracy=0.88; cases=15, controls=18). The limit of quantification (LoQ) for the plasma-based MET test is estimated at ~0.8% ctDNA. Relationship between Precede's cross-validated, ctDNA-corrected MET score and MET FISH copy number **(middle panel)** or MET IHC (% of cells with 3+ staining) **(right panel)**, with samples colored by tissue MET status.

Table 1: ctDNA Detection in AZ NSCLC Plasma Samples with Varying Tissue-based MET Levels

Tissue-based MET status*	No. of plasma samples profiled using Precede assay	No. of evaluable plasma samples for downstream analyses (ctDNA >0.5%)
MET+	15	15
MET-	25	18

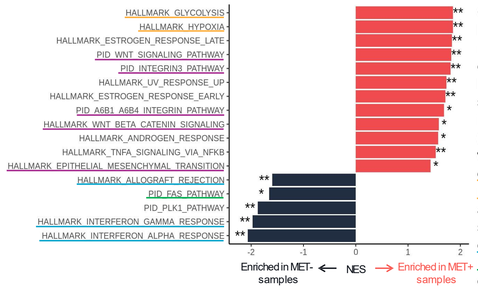
Plasma ctDNA fraction estimated in NSCLC samples stratified by tissue MET status (MET− vs MET). The limit of detection (LoD) is estimated at ~0.5% ctDNA. *Tissue-based analysis of matched post-progression biopsies scored 15/40 tumors as MET-high/+(FISH 10+ and IHC 3+ ≥90%) and 25 samples as MET-low/- (FISH <10, IHC 3+ <90%).

Figure 2: Precede's Comprehensive Epigenomic Assay Enables Genome-wide Exploration of Differences in MET Tissue Positive vs Negative Plasma Samples



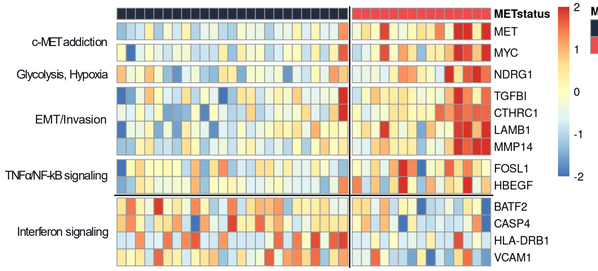
Volcano plot showing differential epigenomic activity between MET+ and MET− plasma samples. The x-axis (mean logFC) represents the mean log fold-change in gene-associated signal between MET+ and MET− samples across epigenomic analytes, while the y-axis shows the $-\log_{10}$(combined p-value). Dots are colored by direction of change (red-higher in MET+ vs black-higher in MET−), with selected genes labeled (combined adjusted p-value <0.1). Extreme logFC values are clipped for visualization.

Figure 3: Distinct Transcriptional Programs are Associated with MET+ vs MET- Plasma Samples



Gene set enrichment analysis (GSEA) of Hallmark and PID pathways using genes ranked by differential combined epigenomic activity between MET+ and MET− samples. Normalized enrichment scores (NES) indicate pathways enriched in MET+ (positive NES) or MET− (negative NES) samples. MET+ samples were enriched for pathways related to glycolysis and hypoxia-adapted metabolism, as well as developmental and invasive programs. In contrast, MET− samples showed enrichment of interferon-driven immune activation and apoptotic priming. Significance thresholds are denoted (*adjusted p<0.1; **adjusted p<0.05).

Figure 4. Representative Differentially Regulated Genes Distinguish MET-associated from MET-independent Programs



Heatmap of combined epigenomic signal for selected genes with differential activity between MET tissue positive and negative samples. Genes are organized by functional categories including c-MET addiction, glycolysis/ hypoxia, EMT/invasion, TNFα/NF-κB signaling, and interferon signaling. Distinct epigenomic patterns separate MET+ and MET− samples: MET+ samples showed higher activity at genes involved in MET-associated oncogenic programs (e.g., *MYC*), invasion and EMT (e.g., *MMP14*, *FOSL1*), and hypoxia/glycolysis (e.g., *NDRG1*), whereas MET− samples showed increased signal at immune-related genes, including interferon pathway components (e.g., *BATF2*, *HLA-DRB1*).

Figure 5: Baseline Samples Do Not Demonstrate Evidence of SCLC Transformation

Predicted probability of small cell lung cancer (SCLC) classification using a previously developed Precede SCLC classifier[2] across AZ NSCLC samples. The dashed line represents the threshold for probability of samples displaying SCLC-like features.

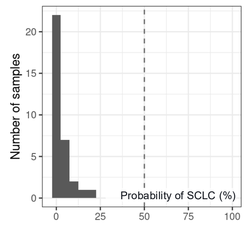
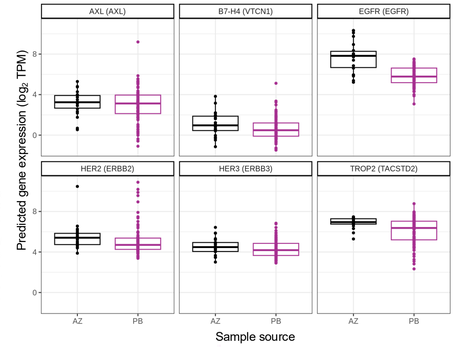


Figure 6: Precede-predicted Gene Expression Identifies Potentially Actionable Markers In Baseline Plasma Samples



**Precede internal cohort is an independent NSCLC dataset (largely EGFR wild-type) included for contextualization of expression distributions and is not directly comparable to, or intended as a control/ reference for, the AZ EGFRm cohort.

Precede-predicted expression scores for select genes (*AXL, B7-H4, EGFR, ERBB2, HER3, TROP2*), derived using Precede's proprietary algorithms to infer gene expression from plasma, in AZ EGFRm NSCLC samples (AZ; black dots), and a cohort of Precede internal NSCLC samples** (PB; purple dots). Boxplots with individual data points show distributions across sample sources (ctDNA >3%). One AZ sample showed elevated *ERBB2* (HER2) signal consistent with amplification; this was confirmed by baseline ctDNA NGS demonstrating two independent *ERBB2* hotspot mutations (S310F/S305F) in addition to *ERBB2* amplification. Consistent with this genomic profile, efficacy outcomes (response, PFS) in this patient were poor. These results illustrate robust detection of heterogeneous expression of therapeutically relevant targets and resistance-associated markers across EGFRm and EGFR wild-type samples and demonstrate that plasma-based inference can capture biology consistent with tissue findings.

CONCLUSIONS

- An exploratory study leveraging comprehensive epigenomic profiling from 1mL of plasma enabled a MET test that demonstrated high concordance with MET status based on tissue IHC and FISH in EGFRm NSCLC (AUC 0.97; balanced accuracy 0.88; N=40).
- This assay, also integrating ctDNA detection and quantification, identified differential activation of biologically distinct programs, including metabolic and invasive signaling in MET+ samples vs immune-related activity in MET- samples.
- Additionally, baseline plasma profiling enabled assessment of additional targets beyond MET status, including the ability to assess lineage plasticity (e.g., SCLC-like transformation), and identified therapeutically actionable targets.
- While current standard-of-care assessment of MET activation in patients progressing on 1L EGFR TKIs remains tissue-based (IHC/FISH), this approach provides an accessible and scalable blood-based test to increase identification of patients with NSCLC post-EGFR inhibitor treatment who may benefit from MET-targeted therapy, monitor resistance, and inform future combination or sequential MET-directed strategies, particularly in settings where repeat tissue sampling is infeasible or limited.

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Contact: J. Carl Barrett, PhD
Precede Biosciences
Email: carl.barrett@precede.bio

References: [1]Ahn et al., *J Thorac Oncol.*; 17, S469-S470 (2022).
[2]Guess et al., *Proceedings of the AACR Annual Meeting* (2024).

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