Plasma Epigenomic Profiling Identifies Mechanisms of Sensitivity and Resistance to Tepotinib in *MET*ex14 Skipping Metastatic NSCLC

Sunny Das,[1] Robert Fisher,[1] Aparna Gorthi,[1] Vy Tran,[1] Tyrone Tamakloe,[1] Charlene O'Brien,[1] Hat Sawaengsri,[1] Kyle Gowen,[1] Kristian Cibulskis,[1] Humphrey Gardner,[1] Corrie Painter,[1] Anthony D'Ippolito,[1] Matthew Eaton,[1] Andreas Kloetgen,[2] Andreas Machl,[3] Zheng Feng,[3] Christopher Stroh,[2] Carl Barrett[1] | [1]Precede Biosciences, Boston, MA, United States; [2]The healthcare business of Merck KGaA, Darmstadt, Germany; [3]EMD Serono, Billerica, MA, United States

BACKGROUND

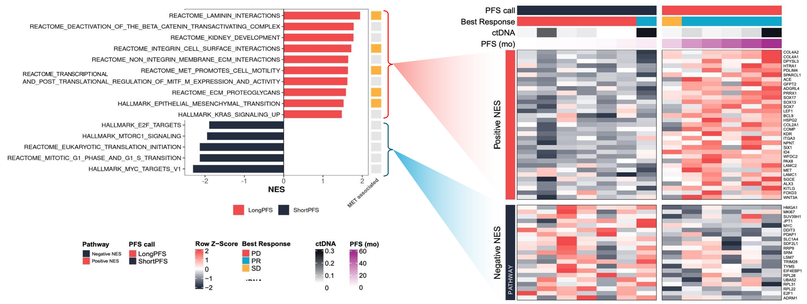
- *MET* exon 14 skipping mutation (*MET*ex14) is an actionable biomarker in a subset (2-4%) of patients with advanced/ metastatic NSCLC[1].
- Tepotinib is an approved oral, highly selective MET tyrosine kinase inhibitor (TKI) for these patients[2].
- However, clinical responses remain heterogeneous within patients harboring this genomic alteration, suggesting that additional biological features beyond *MET*ex14 skipping influence therapeutic sensitivity[3].
- Moreover, resistance to MET inhibition prior to treatment or following tepotinib therapy emerge dynamically, and repeated tissue sampling is challenging.
- To overcome these limitations and identify transcriptional programs associated with sensitivity and resistance to tepotinib, we applied a comprehensive epigenomic liquid biopsy platform using 1mL of plasma to baseline and longitudinal samples collected from a subset of patients with *MET*ex14 skipping NSCLC enrolled in the Phase II VISION study.

METHODS

- A total of 144 baseline, on-treatment (OT), and end-of-treatment (EOT) samples from patients with *MET*ex14 skipping NSCLC enrolled in the VISION study (NCT02864992) were profiled using a comprehensive epigenomic assay (Precede Biosciences, Boston, MA) from 1mL of plasma for downstream analysis, along with downstream analyses to infer genome-wide transcriptional activity from cell-free DNA in plasma samples (Precede Bio Insight™).
- Pathway analyses using proprietary assessment of genome-wide, ctDNA-corrected differential comprehensive epigenomic activity over healthy background between progression-free survival (PFS)-stratified baseline responders (top tertile PFS) vs non-responders (bottom tertile PFS) were used to identify mechanisms of intrinsic resistance to tepotinib.
- SCLC/neuroendocrine transformation was assessed in all samples using the previously developed Precede SCLC classifier[4]. *MET* copy number was assessed using the ichorCNA algorithm[5].

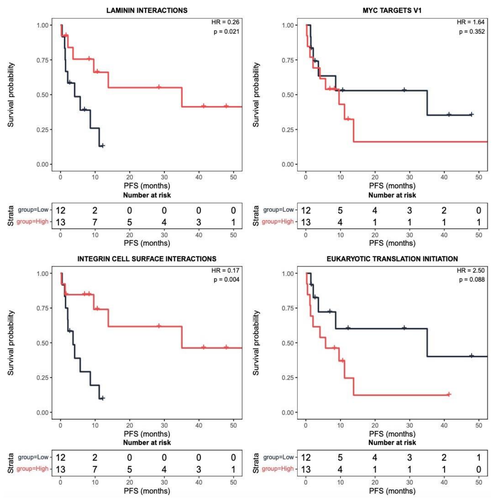
RESULTS

Figure 1: Comprehensive Baseline Plasma Epigenomic Profiling Identifies Transcriptional Pathways Distinguishing Non-responders from Responders to Tepotinib Despite Shared *MET*ex14 Skipping Mutations



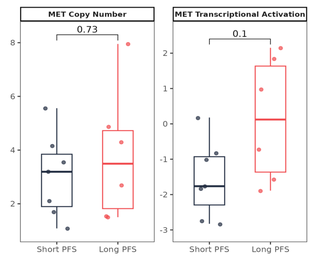
Gene set enrichment analysis (GSEA) comparing baseline epigenomic profiles between responders (top tertile PFS) and non-responders (bottom tertile PFS) to tepotinib. Analyses shown here were limited to baseline samples meeting pre-specified inclusion criteria: ctDNA ≥3%, analyte-level QC pass, evaluable CBOR per iRECIST, and available PFS annotation; from this subset, only the top and bottom PFS tertiles were included (short PFS, n=7; long PFS, n=6). **(Left)** Bar plot shows normalized enrichment scores (NES) for top-10 significantly enriched pathways (FDR<0.05). Responders (red bars; positive NES) exhibited enrichment of pathways associated with MET-signaling dependence (yellow), including integrin signaling, extracellular matrix (ECM) remodeling and invasion. In contrast, non-responders (black bars; negative NES) showed enrichment of proliferative and translational programs, including MYC-associated activity, and metabolic pathways. **(Right)** Heatmap of epigenomic signal (z-scored across samples) for leading-edge genes derived from significant pathways identified in differential analysis. Samples are annotated by PFS category, best response, ctDNA fraction, and PFS duration. The displayed genes capture pathway-level differences between responders and non-responders, with coordinated activity in genes linked to MET-associated signaling programs and ECM remodeling in responders vs genes linked to proliferative signaling in nonresponders.

Figure 2: Patient-specific Pathway Activity Scoring Stratifies Patients by Outcome and Reveals MET-dependent vs Independent Biology Beyond *MET*ex14 Skipping Mutations at Baseline



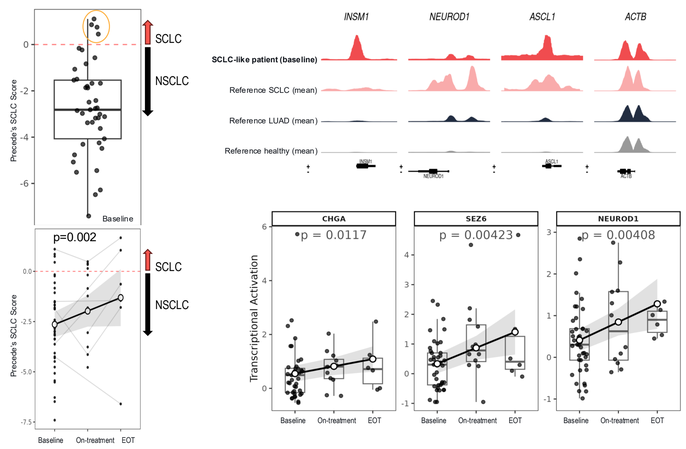
Kaplan-Meier curves of PFS stratified by high (red) vs low (black) pathway activity scores for representative pathways associated with response (e.g., laminin interactions, integrin cell surface interactions) or resistance (e.g., *MYC* targets, eukaryotic translation initiation). Pathway activity scores were computed per sample using gene set variation analysis (GSVA) on epigenomic signals and dichotomized at the median (baseline QC-pass samples, ctDNA >3%, N=25; censored patients indicated). Patient-level pathway activity scores associated with clinical outcome, reflecting distinct programs linked to improved vs poorer PFS. Notably, these signals captured both MET-dependent and MET-independent biology, explaining heterogeneous responses despite shared *MET*ex14 skipping mutations.

Figure 3: *MET* Transcriptional Activation, But Not Copy Number, Trends with Response in Baseline *MET*ex14 Skipping NSCLC



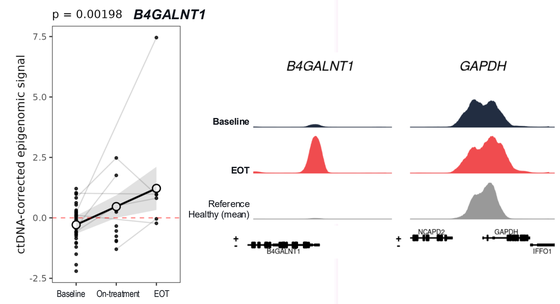
Distribution of *MET* copy number (left boxplot; estimated using ichorCNA) and *MET* transcriptional activation scores (right boxplot) in baseline plasma samples across response groups defined by tertile PFS (short PFS vs long PFS). Analyses shown here were limited to baseline samples meeting pre-specified inclusion criteria: ctDNA ≥3%, analyte-level QC pass, evaluable CBOR per iRECIST, and available PFS annotation; from this subset, only the top and bottom PFS tertiles were included (short PFS, n=7; long PFS, n=6). Boxplots display median and interquartile range with individual data points. *MET* copy number showed no association with response, whereas *MET* transcriptional activation demonstrated a trend toward higher activity in patients with longer PFS, suggesting a closer relationship with clinical outcome. Neither comparison reached statistical significance. Some *MET*-amplified samples were not included due to QC failure or censoring.

Figure 4: Longitudinal Plasma Epigenomic Profiling Reveals Baseline and Treatment-associated Increases in Neuroendocrine Features Consistent with Lineage Plasticity



Jittered box plot (top left): At baseline, a subset of samples (dots within yellow circle) exhibited SCLC-like epigenomic signal (scores >0; dotted red line), indicating neuroendocrine differentiation (SCLC-like) features, assessed using a previously developed Precede SCLC classifier[4]. Analyses shown here were limited to samples meeting pre-specified inclusion criteria: ctDNA >1% (SCLC classifier LoD) and analyte-level QC pass (N=59 across all timepoints). **Signal tracks (top right):** Promoter tracks show elevated signal at key neuroendocrine genes *(ISNM1, NEUROD1, ASCL1)* in one of the predicted SCLC-like samples from the baseline cohort compared to mean signals from Precede's internal reference cohorts of known SCLC, LUAD and healthy samples. *ACTB* is a representative housekeeping gene. **Jittered strip plot (bottom left):** SCLC scores increased over time in patient-matched longitudinal samples (OT and EOT; grey lines; p=0.002, linear mixed-effects model), consistent with emergence of neuroendocrine differentiation during tepotinib therapy. The fixed-effect estimate of mean SCLC score across timepoints is denoted by the black line connecting white dots and the accompanying grey zone denotes the associated confidence intervals. **Jittered strip plot by gene (bottom right):** Transcriptional activation of representative neuroendocrine genes significantly increased over time in patient-matched longitudinal samples.

Figure 5: Treatment-associated Increase in GD2 Synthase *(B4GALNT1)* Epigenomic Activity Highlights an SCLC-associated Actionable Vulnerability in *MET*ex14 Skipping NSCLC



B4GALNT1 (encoding GD2 synthase) is a glycosyltransferase that promotes cancer cell growth, stemness, metastasis, and immune evasion, and is an emerging ADC/ CAR-T target in multiple cancers[6], including SCLC[7]. **Jittered strip plot (left):** Changes in epigenomic signal for *B4GALNT1* across treatment timepoints. Grey lines connect patient-matched samples. A linear mixed-effects model demonstrated a significant increase in activity with treatment (p=0.00198), indicating induction of GD2 synthase-associated programs during acquired resistance to tepotinib. ctDNA-corrected epigenomic signal at *B4GALNT1* for the patient with samples at all timepoints is denoted by black line connecting white dots and the accompanying grey zone denotes the associated confidence intervals. Analyses shown here were limited to samples meeting pre-specified inclusion criteria: ctDNA >1% and analyte-level QC pass (N=59 across all timepoints). **Signal tracks (right):** Promoter tracks show elevated signal at *B4GALNT1* in an EOT sample compared to its paired baseline, along with mean signal from Precede's internal reference cohort of healthy samples. *GAPDH* is a representative housekeeping gene.

CONCLUSIONS

- Comprehensive epigenomic profiling of baseline plasma samples revealed functional heterogeneity within *MET*ex14 skipping NSCLC, distinguishing responders with MET-dependent signaling from those with intrinsic resistance to tepotinib.
- Functional activation of MET signaling, characterized by epigenomic activity of MET-associated pathway genes, rather than *MET* copy number, stratified response, highlighting a potential opportunity to enrich for patients most likely to benefit from tepotinib.
- Baseline and longitudinal profiling showed increase in lineage plasticity and neuroendocrine (SCLC-like) features, and induction of GD2 synthase *(B4GALNT1),* revealing a potential actionable vulnerability using anti-GD2 ADC or CAR-T therapy.
- These findings support the use of our blood-based test to guide patient selection, monitor resistance longitudinally, and inform combination or sequential therapeutic strategies in *MET*ex14 skipping NSCLC, particularly where repeat tissue sampling is infeasible or limited.

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

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