KALC 2022
Korean Association for Lung Cancer International Conference
November 10-11, 2022 | Lotte Hotel World, Seoul, Korea

Pilot Untargeted Blood Plasma Metabolite Profiling of Tyrosine Kinase Inhibitor Response in Filipino Non-Small Cell Lung Cancer (NSCLC) Patients

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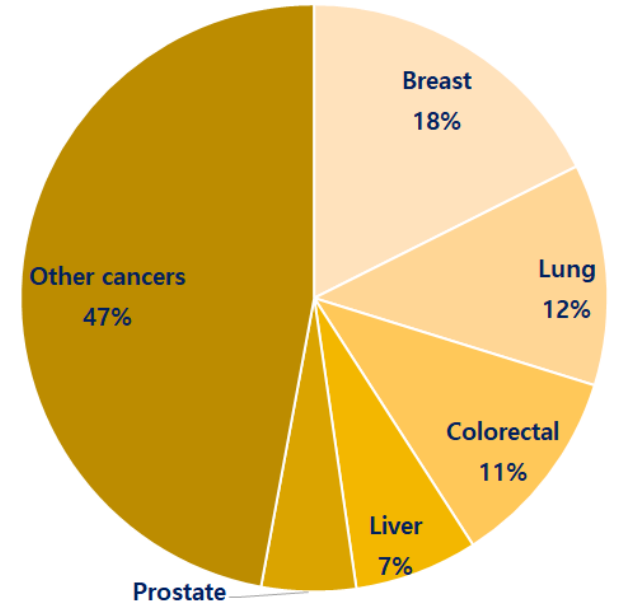
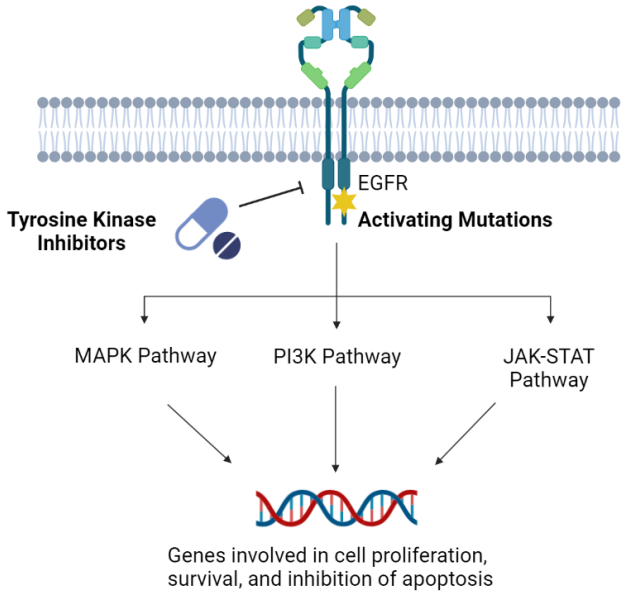
INTRODUCTION





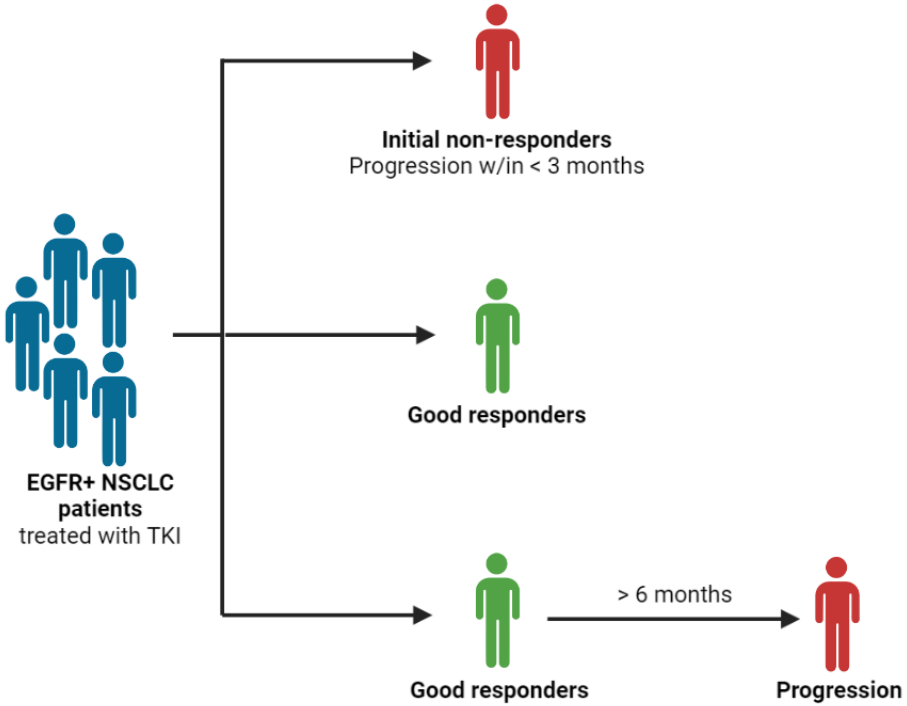
Lung cancer was the 2nd most diagnosed cancer in the Philippines in 2020, accounting for 12% of all cases. NSCLC accounts for about 85% of lung cancer cases.

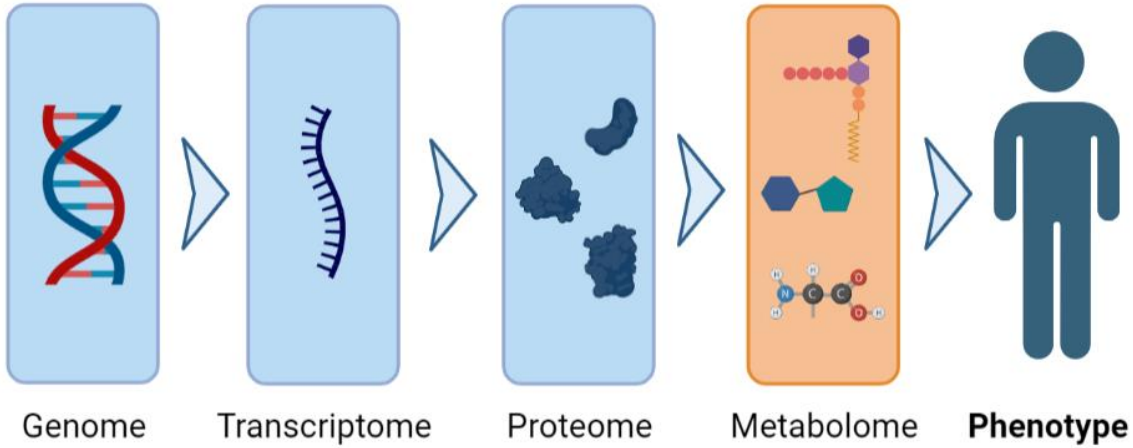
In the Philippines, 42.4 - 49.4% of NSCLC cases have mutations in the EGFR gene, which is higher than the global average frequency of 11.9%. **EGFR mutations drive cell malignancy through the activation of downstream cell proliferation pathways.**

Advances in molecular medicine have led to the development of **EGFR Tyrosine Kinase Inhibitor (EGFR-TKI) drugs that target EGFR-mutation-positive NSCLC.**

GLOBOCAN, 2020

INTRODUCTION





Patients undergoing EGFR-TKI therapy still exhibit resistance after months of treatment. There is a need to evaluate drug response in NSCLC to improve clinical outcomes

Beyond genomics, transcriptomics, and proteomics, **metabolomics has garnered attention in recent years as a tool in biomarker discovery.** Metabolites are the end-products and intermediates in biological processes and provide a comprehensive snapshot of the processes that have led to biological phenotype.

Since cancer is a disease with significantly altered metabolism, early-stage malignancy may be detectable through monitoring metabolic changes in cells.

Previous studies have shown that **metabolomics has the potential to predict anti-cancer drug response, and distinguish good responders vs. poor responders**

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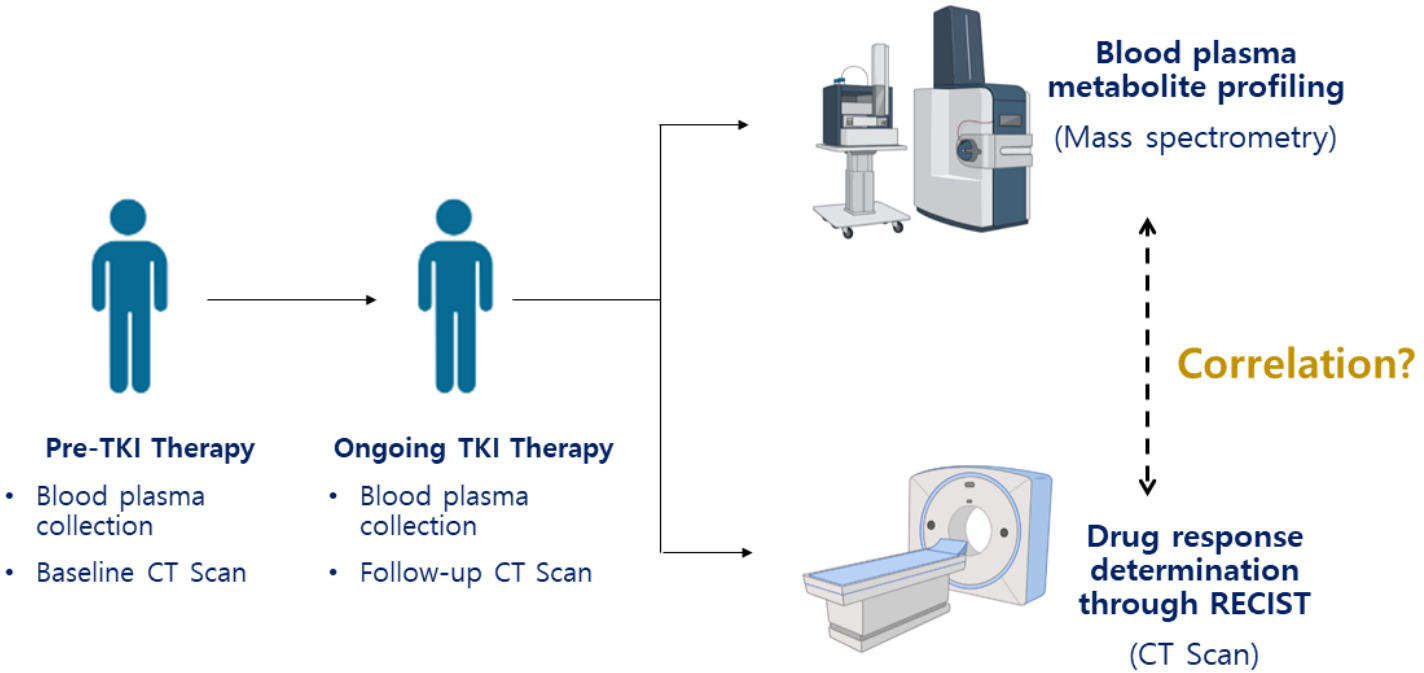
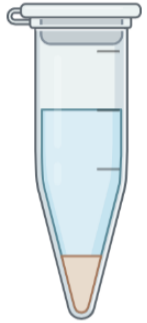
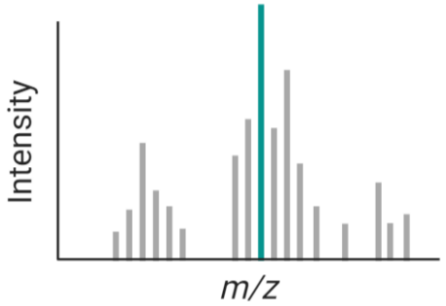
OBJECTIVES

- Characterize blood plasma metabolites profiles of NSCLC patients undergoing TKI therapy through an untargeted metabolomics approach
- Determine correlations of patient-metabolite profiles with specific Response Evaluation Criteria in Solid Tumors (RECIST) values
- Compare metabolite profiles of patients responsive to EGFR-TKI and those with progressive disease



METHODOLOGY





Blood Plasma Collection

- Blood plasma was collected from NSCLC patients, processed through centrifugation, and stored at -80°C until metabolite extraction

Metabolite Extraction

- Metabolites were extracted from blood plasma using methanol as solvent
- Oleic acid and 1-(4-fluorobenzyl)-5-oxoproline were used as internal reference standards

Mass Spectrometry

- The metabolite profile was analyzed using a Waters Xevo G2-XS QToF mass spectrometer
- Samples were analyzed both in positive and negative ionization modes

Analysis

- Spectral processing, enrichment, and functional analyses were all performed using the MetaboAnalyst 5.0 online software

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PATIENT AND SAMPLE PROFILES

Table 1. Patient profiles

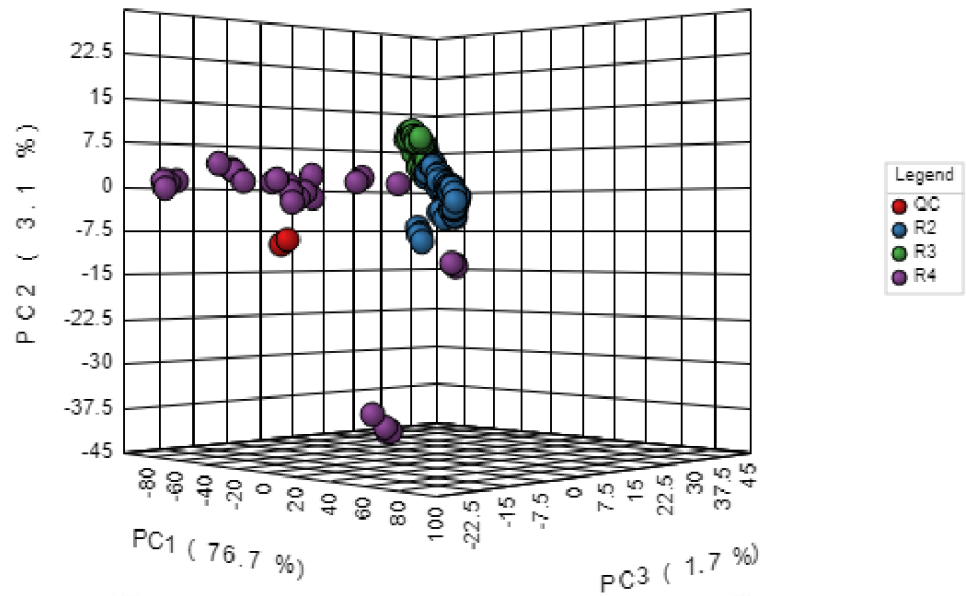
Characteristic	Overall (n=17) (%)
Age	
Median	63
Mean	61.41
Range	38 - 77
Sex	
Female	13 (76.47)
Male	4 (23.53)
Smoking Status	
Nonsmoker	10 (58.82)
Firsthand	1 (5.88)
Secondhand	6 (35.29)
Stage	
III	1 (5.88)
IV	16 (94.12)
EGFR Status	
Exon 21 L858R	6 (35.29)
Exon 19 Deletion	10 (58.82)
Exon 21 L861Q	1 (5.88)

Table 2. Sample profiles

Drug Response	RECIST no.	No. of samples
Sensitive	2 (Partial response)	17
	3 (Stable disease)	13
Progressive	4 (Progressive)	7
TOTAL		**37**

RESULTS

A



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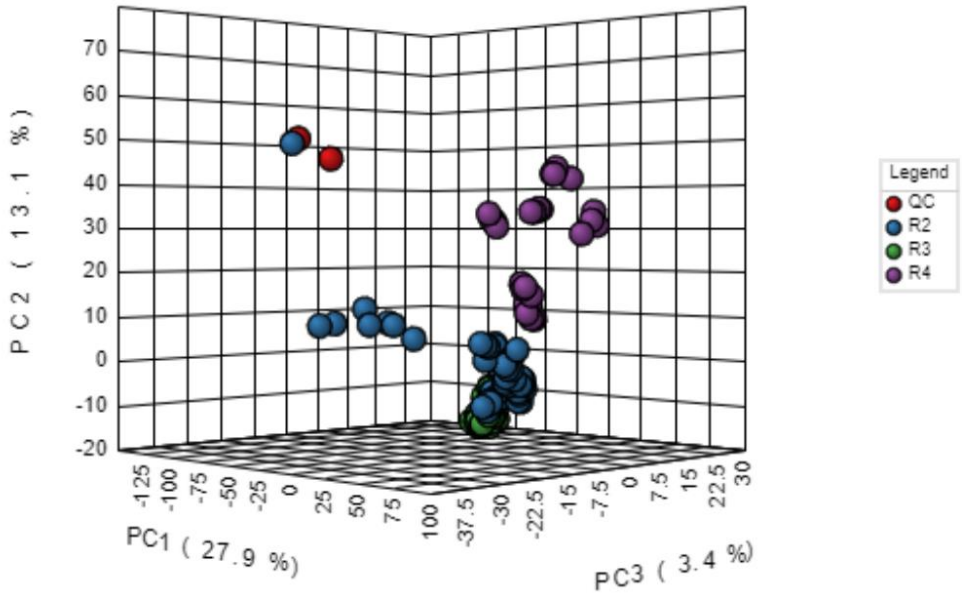

Figure 1. Unsupervised principal component analysis (PCA) of Filipino NSCLC Patients undergoing EGFR-TKI Therapy.

Clustering of metabolite profiles according to the degree of drug response is observed in both positive (A) and negative (B) ionization modes. RECIST values (R2, R3, R4) correspond to partial response, stable disease, and progressive disease, respectively. Spectral processing, metabolite identification, and unsupervised PCA were performed using MetaboAnalyst 5.0.

RESULTS

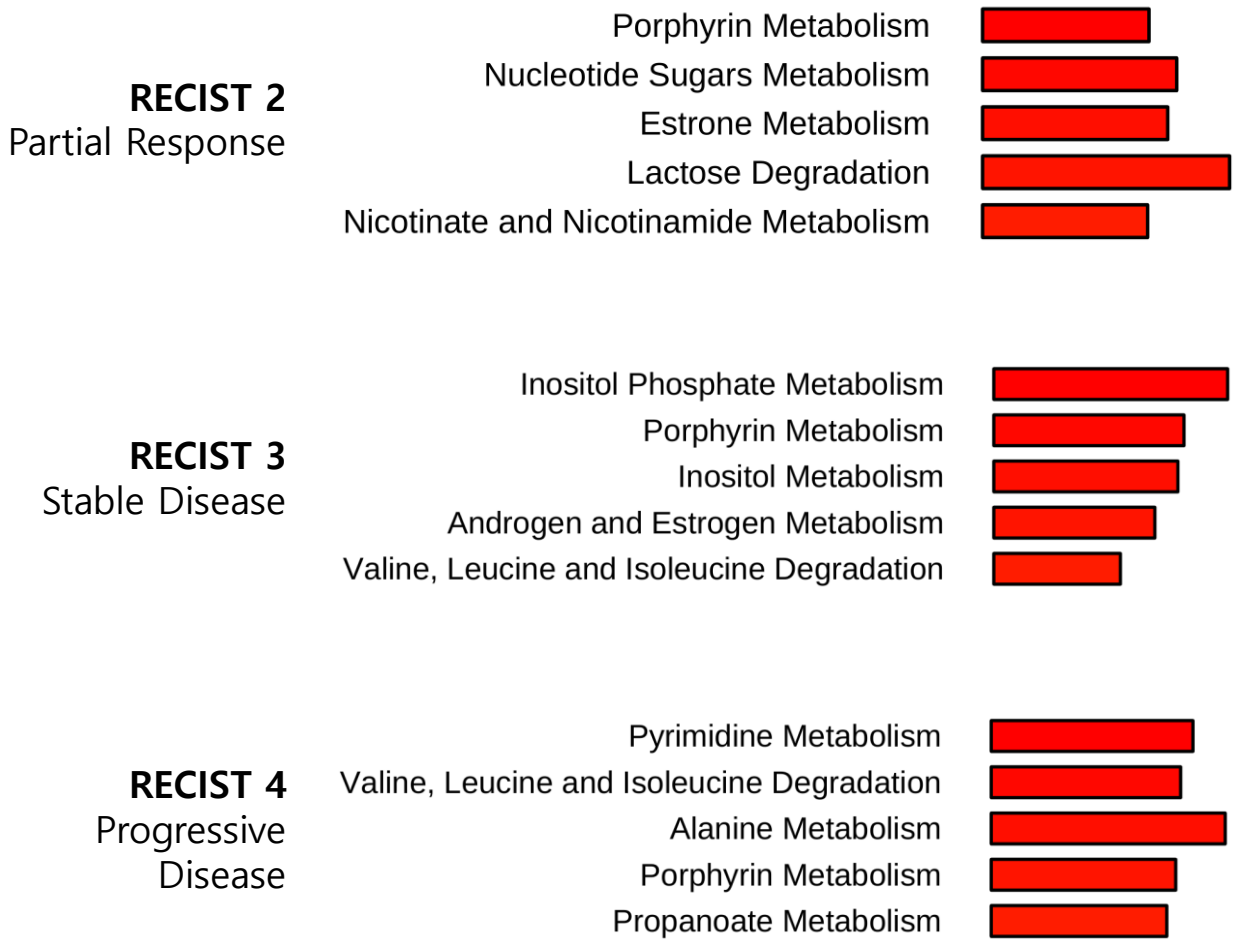

Figure 2. Metabolite Set Enrichment Analysis (MSEA) of metabolite profiles corresponding to each RECIST value.

The top 5 enriched metabolite sets for each RECIST value for the positive ionization mode is shown. Interestingly, **pyrimidine metabolism is the most enriched metabolite set in the blood plasma of patients with progressive disease (RECIST 4).** Increased pyrimidine metabolism is a hallmark of cancer, as cancer cells need a continuous supply of dNTPs to maintain their aberrant growth and replication. Dysfunctional pyrimidine metabolism has also been previously linked to chemotherapy resistance.

MSEA was performed using MetaboAnalyst 5.0. Metabolites without putative annotations were not included in the analysis.

RESULTS

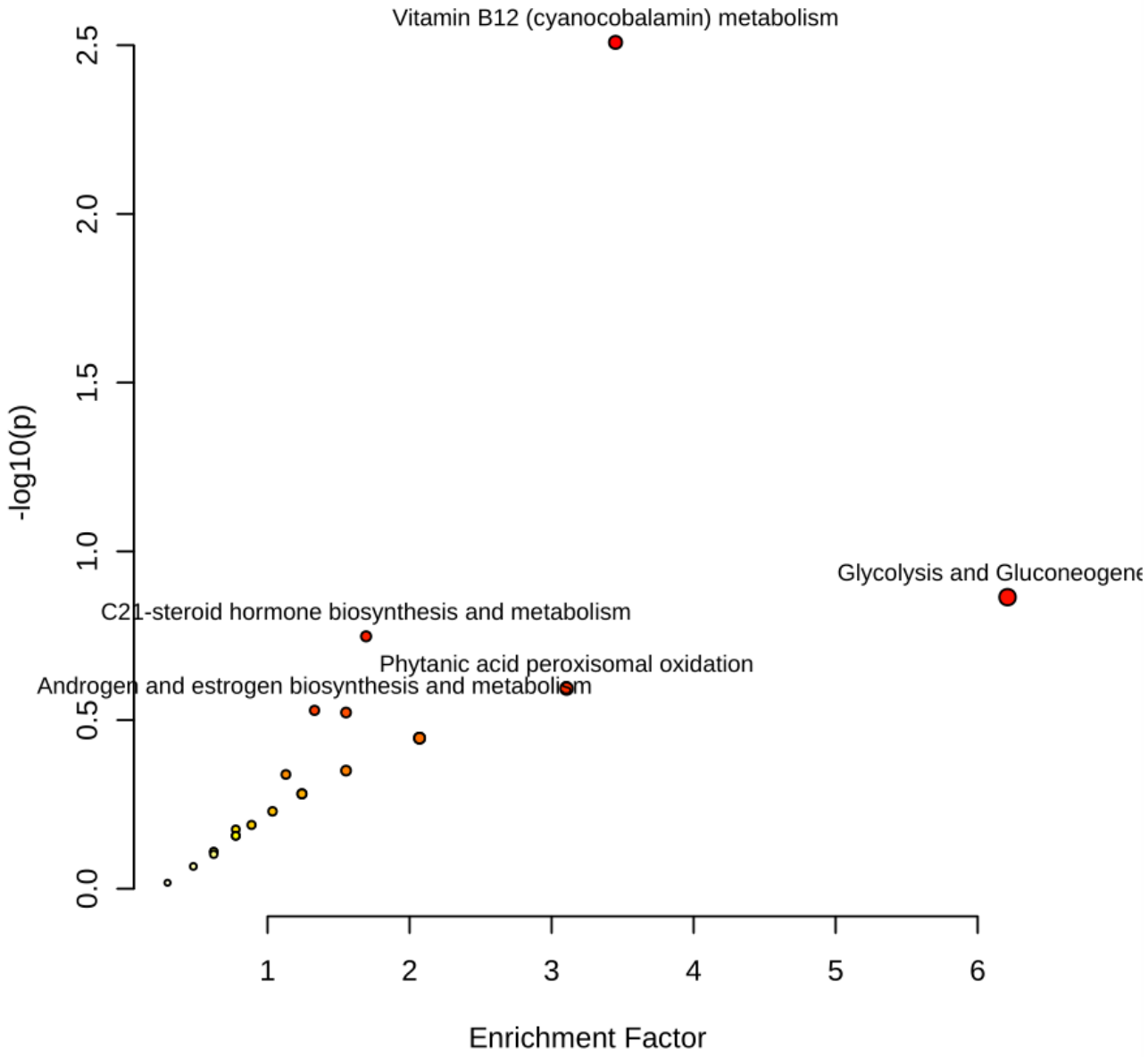

Figure 3. Functional analysis of sensitive and progressive metabolite profiles.

RECIST 2 and 3 (sensitive) metabolite profiles were combined and compared with RECIST 4 (progressive) profiles. Vitamin B12 metabolism, as well as the glycolysis and gluconeogenesis pathways were found to be the most enriched. Previous studies have shown that persistent vitamin B12 elevation in blood plasma is linked to solid cancer incidence, while metabolic perturbations in the glycolysis and gluconeogenesis pathways are a hallmark of cancer.

The mass-to-charge ratio (m/z) and retention time values were ranked according to p-value (cutoff was set to <0.05) and analyzed through the Mummichog algorithm through MetaboAnalyst 5.0. Only the positive ionization mode was analyzed.

CONCLUSION

Metabolomics has been used in previous studies to profile and identify potential biomarkers for oncogenic drug response. The results of this study suggest that **patients undergoing EGFR-TKI therapy with progressive disease possess a distinctive blood plasma metabolite signature that may be used to distinguish them from responsive patients.** Further statistical analysis is currently underway to determine the stratification potential of the identified metabolite features.

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ACKNOWLEDGEMENTS

The researchers would like to thank the Department of Science and Technology and the Philippine Council for Health Research and Development for the funding.