Non-Interventional Study Protocol
C4221028

OCEANMIST - Comparative effectiveness of different targeted therapies for BRAF-mutated unresectable/metastatic melanoma in the United States

Statistical Analysis Plan
(SAP)

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1 AMENDMENTS FROM PREVIOUS VERSION(S)

Significant updates have been made to the data source and analytical approach compared to the original OCEANMIST statistical analysis plan. Below, we provide a high-level overview of the key differences with additional details described below.

Data Source Updates

- Analyses conducted based on the originally submitted protocol and analysis plan for study C4221028 were limited to descriptive and unadjusted analyses of real-world data from Flatiron Health, as the sample size of the identified encorafenib plus binimetinib study cohort was judged to be too small for conduct of adjusted comparative effectiveness analyses. In this amended analysis plan, data for encorafenib plus binimetinib patients will be pooled from the phase 3 COLUMBUS trial and the Flatiron Health real-world database to maximize sample size. This pooling is justified based on the empirical consistency in OS outcomes across COLUMBUS and Flatiron Health after harmonizing key patient selection criteria, adjusting for differences in baseline characteristics, and imputing missing data on baseline characteristics (HR = 0.94, 95% CI: 0.43, 1.45), as described in study C4221035.

Analytical Approach Updates

- To ensure identification of patient populations in Flatiron that are comparable to patients in the COLUMBUS trial, key patient selection criteria related to age, ECOG performance score, history of brain metastasis and prior use of immunotherapy and targeted therapies from the COLUMBUS trial will be applied to all treatment groups in Flatiron Health.

- To ensure that representation of each targeted therapy from the real-world data was as comprehensive as possible and that patients were followed from treatment initiation consistent with the COLUMBUS trial, patients included in each treatment cohort will be required to have index dates (i.e., treatment initiation dates) that were on or after the dates of FDA approval of that targeted therapy in metastatic melanoma (specifically, 09 January 2014 for DAB+TRAM, 10 November 2015 for VEM+COBI and 27 June 2018 for ENCO+BINI). This is in contrast to the original approach, which defined the follow-up period for all cohorts as beginning from 20 November 2015. This could have led to the exclusion of some patients initiating DAB+TRAM before approval, and could potentially inadvertently include off-label usage of ENCO+BINI.

- For the real-world cohorts, patient baseline characteristics will be obtained from visits ≤3 months prior to the index date, with data from visits closest in time to the index date being used as available. In contrast, in the original analysis plan, to maximize availability of baseline characteristics, the 'baseline' period was allowed to include visits up to 6 months prior to treatment initiation. The decision

to use a stricter definition of baseline here was based on clinical input that certain baseline characteristics of interest (e.g., ECOG PS) are best assessed closest to the index date, despite potential for increased missingness. To address missingness, imputation of baseline characteristics was conducted as described below.

- Key clinical characteristics like ECOG and LDH were missing at baseline for 40-60% of patients in the original study cohort, which would have hampered ability to adjust for potential confounding due to these factors. To address missingness, in this analysis plan we will impute data using multiple imputation by chained equations prior to conducting adjusted analyses. Imputation models will be based on baseline characteristics that would be considered in adjusted models for OS.

- Inverse probability of treatment weighting without any imputation was proposed in the original study design to account for baseline differences. Instead, we will use multivariable Cox proportional hazards models fit to each of the imputed dataset and combine results across all imputations. This approach is preferred as it allows us to assess how included covariates are associated with OS in our final models, in addition to estimating differences between the treatments of interest.

- Comparisons of OS and PFS will be stratified by first-line and second-line of therapy in the original study design. Analyses conducted in this revised analysis plan will not be stratified by line of therapy to allow for a larger sample size to compare OS and PFS across targeted therapy treatment cohorts. A line of therapy variable will be included in Cox proportional hazard models, which can facilitate future analyses of comparative effectiveness by line of therapy if required.

2 INTRODUCTION

Melanoma, caused when malignant cancer cells form in melanocytes, is the fifth most common cancer in the United States (US) and the deadliest form of skin cancer.[1] Incidence of melanoma has been increasing over recent decades, with the overall incidence rate of 229.1 cases per million person based on recent estimates. Based on American Cancer Society estimates, there were an estimated 99,780 new cases of melanoma and ~7,650 deaths from melanoma in the US in 2022.[1-3]

Approximately 9% of melanoma patients are diagnosed with regional spread to lymph nodes and 4% are diagnosed with metastatic disease.[1] Compared to patients with localized melanoma, patients with metastatic melanoma have poorer prognosis and worse outcomes, which can be further worsened by the presence of v-Raf murine sarcoma viral oncogene homolog B protein (BRAF) mutations.[4] The BRAF protein plays an important role in normal cell growth, proliferation, differentiation, and survival.[5] The presence of BRAF V600 mutations, found in ~40–60% of melanoma cases, can lead to sustained mitogen-activated protein kinase (MAPK) kinase (MEK) pathway signaling, resulting in tumor growth and progression.[6]

Recommended treatment options for metastatic melanoma include immunotherapy (IO), which attempts to stimulate host responses to effectuate tumor destruction, and targeted therapies (TT), which inhibit molecular pathways to prevent tumor growth and maintenance.[7] The discovery of targeted therapy as a treatment for melanoma has emerged as a milestone development in oncological research.[8] In 2018, the US Food and Drug Administration (FDA) approved the use of encorafenib (BRAFTOVI®) in combination with binimetinib (MEKTOVI®) for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation (as detected by an FDA-approved test) based on the pivotal phase 3 COLUMBUS trial.[9, 10] This accompanied existing BRAF-/MEK-inhibitor therapies including dabrafenib plus trametinib (FDA approval 09 January 2014) and vemurafenib plus cobimetinib (FDA approval 10 November 2015).

There is increasing interest in evaluating comparative effectiveness of BRAF-/MEK-inhibitor therapies. In a recent study, we showed that overall survival (OS) for metastatic BRAFV600-mutant melanoma patients was similar across the COLUMBUS trial and real-world practice data from the Flatiron Health database (Hazard Ratio [HR] = 1.03, 95% Confidence Interval [CI]: 0.53, 1.54; p=0.90) after harmonizing key patient selection criteria, adjusting for differences in baseline characteristics, and imputing missing data on baseline characteristics. Given this empirical consistency in OS outcomes, pooling patients across these settings can be justified and allows for a larger sample size for assessment of comparative effectiveness of encorafenib + binimetinib versus other targeted therapies. To that end, this study will expand on this prior work and compare OS between patients receiving encorafenib + binimetinib in the pooled Phase 3 COLUMBUS trial and the Flatiron Health Electronic Health Records (EHR) database versus patients receiving other targeted therapies. Baseline profiles of patients will also be compared to characterize differences in patients receiving different therapies and adjustments will be made for differences across treatment groups in baseline factors associated with OS. Missingness for key baseline characteristics will be addressed using a validated multiple imputation approach.[12]

This non-interventional study is not designated as a Post-Authorization Safety Study (PASS) and it is not a commitment or requirement to any regulatory authority.

2.1 STUDY DESIGN

This is a retrospective cohort study comparing OS outcomes between patients with BRAFV600-mutant metastatic melanoma initiating encorafenib plus binimetinib (ENCO+BINI) versus dabrafenib plus trametinib (DAB+TRAM) or vemurafenib plus cobimetinib (VEM+COBI). Comparisons will be adjusted for differences in baseline characteristics across treatment groups, and account for missing data on adjustment factors. PFS will also be investigated as an exploratory endpoint, subject to comparability of PFS outcome assessment between clinical trial and real-world settings.

Study population

The study population will consist of patients with BRAFV600-mutant metastatic melanoma initiating treatment with ENCO+BINI, DAB+TRAM or VEM+COBI. Data for ENCO+BINI will be pooled across COLUMBUS and Flatiron data, while data for DAB+TRAM and VEM+COBI will be from Flatiron data. The number of patients eligible for the study will be determined in accordance with the sample selection conducted per the inclusion and exclusion criteria described in Section 4.

Data source

COLUMBUS trial

COLUMBUS was a two-part, multicenter, randomized, open-label, Phase 3 clinical trial.[10] Part 1 of the trial investigated the effectiveness and safety of three treatment regimens in patients with locally advanced unresectable or metastatic BRAFV600-mutant metastatic melanoma who were treatment naïve or whose cancer had progressed after 1L IO. In Part 1 of the trial, patients were randomized to one of three treatment arms: 1) 450mg once daily encorafenib and 45mg twice daily binimetinib (n=192), 2) 960mg twice daily vemurafenib (n=191) and 3) 300mg once daily encorafenib (n=194). Randomization was stratified by AJCC disease stage (IIIB, IIIC, IVM1a, IVM1b, or IVM1c), ECOG performance status (0, 1), and BRAFV600 mutation profile (V600E, V600K). Progression-free survival was the primary endpoint in the trial, while OS was one of the secondary endpoints. Patients were enrolled in Part 1 of the trial between 30 December 2013 through 10 April 2015.

The ENCO+BINI arm of Part 1 of the trial will be used in this study (database lock date: 15 September 2020) and combined with the ENCO+BINI cohort from Flatiron EHR identified below. Part 2 of the trial compared encorafenib 300mg once daily plus binimetinib 45mg twice daily versus encorafenib 300mg once daily alone and will not be considered.

Flatiron Health EHR

The Flatiron Health EHR database is a longitudinal, de-identified real-world database derived from EHRs collected in US cancer clinics. The database covers more than 2.6 million active cancer patients treated at over 800 sites of care across 4 US census regions. Patient-level data includes both structured (diagnosis, demographics, laboratory values, biomarker information, drug orders, visits, etc.) and unstructured (physician notes, radiology, and pathology reports, etc.) sources. Data on death is drawn from structured or unstructured data fields in the EHR, and publicly available sources of mortality including the US Social Security Death Index, and commercial obituary data.[13]

Pfizer has contracted with Flatiron for a custom data extract containing information on patients with advanced melanoma who received treatment with BRAF- or MEK-inhibitor therapies. Patients meeting the eligibility criteria defined for this study will be obtained from this custom data extract and used in all analyses.

Treatment/cohort labels

The analysis will focus on the following **targeted therapy regimens**:

- Encorafenib + binimetinib (ENCO+BINI) , pooled across COLUMBUS and Flatiron EHR
- Dabrafenib + trametinib (DAB+TRAM), from Flatiron EHR
- Vemurafenib + cobimetinib (VEM+COBI), from Flatiron EHR

2.2 STUDY OBJECTIVES

Primary Objective

To compare OS between patients with metastatic BRAFV600-mutant melanoma initiating ENCO+BINI versus DAB+TRAM or VEM+COBI

Exploratory Objective

To compare progression free survival (PFS) between patients with metastatic BRAFV600-mutant melanoma initiating ENCO+BINI versus DAB+TRAM or VEM+COBI

This objective is designated as exploratory as, unlike for OS, differences in assessment of PFS between clinical trial data (CTD) and real-world data (RWD) settings may exist that preclude valid comparisons of this outcome across these settings. Suitability of comparative analyses of PFS will be determined following additional exploration to characterize number and frequency of assessments of PFS, and further analyses of comparability of PFS outcomes across trial and real-world settings.

3 HYPOTHESES AND DECISION RULES

3.1 STATISTICAL HYPOTHESES

This study includes the following **null hypotheses** to be tested after adjustment for imbalances between groups (see Section 7 for details on adjustment methods):

1. OS does not differ between patients with BRAFV600-mutant metastatic melanoma initiating treatment with ENCO+BINI versus DAB+TRAM or VEM+COBI.

2. PFS does not differ between patients with BRAFV600-mutant metastatic melanoma initiating treatment with ENCO+BINI versus DAB+TRAM or VEM+COBI.

3.2 STATISTICAL DECISION RULES

The sample size of this study is based on data availability during the study period of interest. Therefore this study is not powered for formal hypothesis testing. The nominal alpha level will be 0.05, 2-sided. No adjustments for multiple comparisons will be made.

4 ANALYSIS SETS/POPULATIONS

4.1 FULL ANALYSIS SET

All patients meeting the inclusion and exclusion criteria will be included.

Inclusion criteria

Inclusion criteria that will be used to identify patients initiating the targeted therapies of interest are provided separately for the clinical trial and real-world data sources below.

COLUMBUS trial

Key inclusion criteria in the COLUMBUS trial were:

- *Histologically confirmed diagnosis of locally advanced, unresectable, or metastatic cutaneous melanoma or unknown primary melanoma*
- *American Joint Committee on Cancer (AJCC) disease stage of IIIB, IIIC, IVM1a, IVM1b, or IVM1c at trial enrolment*
- *Presence of BRAF V600E or V600K mutation in tumor tissue prior to enrollment*
- *At least 18 years of age at randomization*
- *Eastern Cooperative Oncology Group (ECOG) status of 0 or 1 at randomization*
- *Treatment naive or had progressed on or after previous first-line (1L) immunotherapy at randomization with no prior BRAF- or MEK-inhibitor therapy in the adjuvant setting*

Patients from the COLUMBUS trial included in this analysis will be those patients:

- *Randomized to ENCO+BINI arm of the trial to receive 450mg once daily encorafenib and 45mg twice daily binimetinib combination therapy*

Flatiron Health data

Pfizer has contracted with Flatiron for a custom data extract containing information on patients with advanced melanoma. Eligibility criteria applied to the Flatiron custom data received by Pfizer include:

- *Diagnosed with melanoma based on International Classification of Disease 9th and 10th Revisions (ICD-9: 172.x; ICD-10: C43x, D03x) and ≥2 visits on different days in the Flatiron database on or after January 1, 2011.*

- *Clinically confirmed diagnosis of melanoma with pathologic stages III or IV at initial diagnosis or earlier stage disease with a first locoregional or distant recurrence on or after January 1, 2011.*
- *Age ≥18 years at the time of advanced melanoma diagnosis.*
- *Evidence of ≥1 BRAF positive test result at any time based on laboratory or genetic analysis results.*
- *Treatment with ≥1 BRAF-inhibitor (i.e., encorafenib, dabrafenib, vemurafenib) and treatment with ≥1 MEK-inhibitor (i.e., binimetinib, trametinib, cobimetinib) in 1L or 2L line of therapy (LOT), as defined per Flatiron's LOT business rules.*
- *Earliest LOT containing a BRAF- and MEK-inhibitor ≥3 months prior to data cutoff (defined as 30 September 2021)*

Key inclusion criteria from the COLUMBUS trial will be applied to the Flatiron RWD population to align the populations to the extent possible between the CTD and RWD sources. The sample of patients from the Flatiron Health EHR data for this study will be identified using the following additional criteria applied to the custom data cut received from Flatiron:

- *At least 18 years of age at the index date*
- *Confirmed BRAF V600E or V600K activating mutation reported in the data based on laboratory or genetic analysis results*
- *Treatment-naive or had previous 1L IO at index date in the therapeutic setting, based on review of medication orders or administration prior to the index date.*
- *ECOG status of 0 or 1 at the index date*

The following criteria will be additionally used to identify patients receiving each of the treatments of interest:

- ***ENCO+BINI treatment group:** At least 1 order or administration of ENCO+BINI treatment after the diagnosis of metastatic melanoma and after 27 June 2018 (FDA approval date for ENCO+BINI for use in patients with metastatic melanoma).*
- ***DAB+TRAM treatment group:** At least 1 order or administration of DAB+TRAM treatment after the diagnosis of metastatic melanoma and after 09 January 2014 (FDA approval date for DAB+TRAM for use in patients with metastatic melanoma).*
- ***VEM+COBI treatment group:** At least 1 order or administration of VEM+COBI treatment after the diagnosis of metastatic melanoma and after 10 November*

2015 (FDA approval date for VEM+COBI for use in patients with metastatic melanoma).

The index date in each treatment group will be the date of treatment initiation.

Exclusion criteria

Patients meeting any of the following criteria will not be included in the study:

- *Patients with prior BRAF- or MEK-inhibitor therapy*
- *Patients with ECOG performance status ≥ 2 (at the time of randomization for patients from COLUMBUS, during the baseline period for patients in Flatiron EHR)*
- *Patients with a history of leptomeningeal metastases including brain, spinal cord, or nervous system metastases (based on brain MRI or CT scan with contrast-enhanced brain MRI as the preferred assessment in COLUMBUS, and based on the following diagnostic codes in Flatiron EHR: ICD-9-CM: 198.3, 198.4 or ICD-10-CM: C79.31, C79.32, C79.40, C79.49) prior to index*
- *For RWD patients, concurrent enrollment in a clinical trial.*

4.2 SUBGROUPS

No subgroup analyses are planned.

5 ENDPOINTS AND COVARIATES

Table 1 provides a summary of the exposure (treatment group) and covariates within the study.

Table 1 Exposure and baseline characteristics

Variable	*Operational definition*
Treatment group (ENCO+BINI, DAB+TRAM, VEM+COBI)	*Indicates whether patients initiated ENCO+BINI, DAB+TRAM or VEM+COBI treatment*
Age at baseline (years)	*Patient age; defined at the time of study randomization for COLUMBUS trial data and at the time of treatment initiation for Flatiron EHR*
Sex	*Male, Female, Intersex, or Unknown/Missing*
Race	*Asian, Black, White, Other/Multi-Race, or Unknown/Missing*

Body mass index (BMI) at index	*Defined as weight (in kg) divided by squared height (in m^2)*
ECOG at index	*0, 1, Unknown/Missing; defined during the baseline period of both the COLUMBUS trial data and Flatiron EHR*
BRAF mutation status	*V600E, V600K, or Unknown/Missing*
AJCC disease stage at initial melanoma diagnosis	*0, I, II, III, IV, or Unknown/Missing; defined at initial melanoma diagnosis for COLUMBUS trial data and Flatiron EHR*
AJCC disease stage at treatment initiation	*IIIB, IIIC, IVM1a, IVM1b, or IVM1c; defined at treatment initiation in the COLUMBUS trial data. Not available in the Flatiron EHR*
Lactate dehydrogenase (LDH) at index	*Evaluated in units per liter (U/L) and defined at trial baseline in COLUMBUS trial and during the baseline period in Flatiron EHR*
Time from initial melanoma diagnosis to metastatic disease	*Duration (in months) from melanoma diagnosis to the development of metastatic disease*
Time from metastatic melanoma diagnosis to treatment initiation	*Duration (in months) from metastatic melanoma diagnosis to the initiation of treatment*
Year of treatment initiation	*Calendar year when treatment was initiated*
Region	*Europe, North America, Australia, Other, or Missing*
Treatment experience in the therapeutic setting	*Indicates whether patients received targeted therapy as first-line treatment (e.g., Treatment naïve) or as second-line treatment after a prior line of immunotherapy treatment (e.g., Prior 1L IO therapy)*

Prior systemic therapies in the adjuvant setting	*Indicates whether patients previously received systemic therapy (e.g., prior surgery, radiotherapy, immunotherapy, or chemotherapy) before initiating treatment*
Prior surgery in the adjuvant setting	*Indicates whether patients previously used surgery to reduce/remove tumors as a form of treatment*
Prior radiotherapy in the adjuvant setting	*Indicates whether patients underwent radiotherapy among those receiving prior systemic therapy*
Prior IO or chemotherapy in the adjuvant setting	*Indicates whether patients underwent chemotherapy or immunotherapy among those receiving prior systemic therapy*
Number of LOTs after TT treatment initiation	*Indicates the number of LOTs occurring after treatment initiation (0, 1, ≥2, or Missing)*
Number of organs involved	*Indicates number of organs involved or sites of metastases at baseline (1, 2, or ≥3), with all patients having at least 1 site given diagnosis of metastatic disease.* *This data was collected and reported in COLUMBUS. In Flatiron EHR, it will be derived based on counting the number of distinct sites recorded as having metastases based on ICD codes.*
Presence of lung metastases	*Indicates whether patients had lung metastases.* *This data was collected and reported in COLUMBUS. In Flatiron EHR, it will be derived based on having ICD codes reflecting presence of lung or respiratory organ metastases (ICD-9: 197, 197.1; ICD-10: C78.01, C78.02, C78.1, C78.2, C78.30, C78.39).*
Presence of liver metastases	*Indicates whether patients had liver metastases.* *This data was collected and reported in COLUMBUS. In Flatiron EHR, it will be derived based on having ICD codes reflecting presence of liver metastases (ICD-9: 197.7; ICD-10: C78.7)*
Presence of other metastases	*Indicates whether patients had presence of other metastases (e.g., adrenal glands, bone, digestive tract/system, kidney, lymph nodes, reproductive organs, or unspecified sites).* *This data was collected and reported in COLUMBUS. In Flatiron EHR, it will be derived based on having ICD codes reflecting presence of other*

	metastases (ICD-9: 196, 196.1, 196.2, 196.3, 196.5, 196.6, 196.8, 196.9, 197.4, 197.5, 197.6, 197.8, 198, 198.5, 198.7, 198.89; ICD-10: C77.0, C77.1, C77.2, C77.3, C77.4, C77.5, C77.8, C77.9, C78.4, C78.5, C78.6, C78.80, C78.89, C79.00, C79.01, C79.02, C79.11, C79.51, C79.52, C79.60, C79.61, C79.62, C79.70, C79.71, C79.72, C79.81, C79.89, C79.9)

5.1 EFFICACY/EFFECTIVENESS ENDPOINT(S)

Table 2 provides a summary of efficacy/effectiveness endpoints that will be evaluated within the study.

Table 2 Clinical outcomes of interest for efficacy/effectiveness

Variable	*Operational definition*
OS	*In the COLUMBUS trial data, defined as the time from the date of randomization to the date of death due to any cause; if death is not observed, patients will be censored at the date of last contact or the data analysis cut-off date (e.g., 15 September 2020), whichever occurs first* *In Flatiron EHR, will be defined as the time from the index date to the date of death; patients without a date of death will be censored at their last known activity date (e.g., the last clinical note date) or the end of the follow-up period, whichever occurs first*
PFS	*In the COLUMBUS trial data, defined as the time from the date of randomization to the date of the first documented disease progression or death due to any cause, whichever occurred first; if a patient did not have an event at the analysis cut-off date, PFS will be censored at the date of the last adequate tumour assessment* *In Flatiron EHR, will be defined as the time from the index date to either the date of first disease progression event or death in the absence of progression; patients without disease progression or death will be censored at the last date the patient could have been assessed for progression (e.g., the last clinical note date) or the data analysis cut-off date (e.g., 30 September 2021), whichever occurs first*

6 HANDLING OF MISSING VALUES

In this study, multiple imputation by chained equations (MICE)[15] will be used to impute missing data on baseline characteristics. Briefly, the MICE method imputes data for a missing patient characteristic based on iterative regressions of observed data for that characteristic on observed and imputed values of other baseline characteristics. This

process will be repeated for each characteristic until stable estimates are obtained, and the entire procedure will be repeated so that multiple datasets with imputed values for baseline characteristics are created.

Baseline characteristics to be imputed will include ECOG performance score, LDH, and other clinical or demographic characteristics (e.g., race, BMI, etc.). Additional variables (e.g., age, sex, AJCC diagnosis, time from MM diagnosis to treatment initiation, time from initial diagnosis of melanoma to metastatic disease, number of organs at baseline, metastatic sites, treatments in the therapeutic setting, treatments in the adjuvant setting) will be considered for inclusion in the MICE procedure based on their relevance for adjustment and degree of missingness in the study sample. As recommended in the literature, the MICE approach will be used to create multiple datasets (e.g., 100 datasets) with imputed values, which will be used and combined in subsequent analyses.[15]

Missing data on baseline characteristics will be imputed for all treatment groups. Analyses of baseline characteristics will be repeated for each imputed dataset, and then summarized across all imputed datasets.

7 STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

7.1 STATISTICAL METHODS

Analysis of baseline characteristics
Patient baseline characteristics at or prior to the index visit will be summarized for all treatment groups. Patient characteristics to be summarized will be based on availability in both data sources and clinical input. A list of characteristics expected to be summarized is provided in Table 1.

*Baseline characteristics will be summarized at the index date if measured at that time. In the Flatiron data, for baseline characteristics that are not measured at the index date, the closest available measure to the index date within a baseline period will be used. Informed by clinical input and considerations of data availability, the **baseline period** will be defined as a 3-month time window prior to and including the index date.*

Patient baseline characteristics will be summarized descriptively using mean (with standard deviation) and median (with interquartile range) for continuous variables and count (with proportions) for categorical variables. Missingness for each baseline characteristic (at the index date or within the baseline period) will also be summarized.

Unadjusted comparison of OS between treatment groups
OS will be defined as described in Section 8.3. OS will be summarized for each treatment group (i.e., ENCO+BINI [pooled across COLUMBUS and Flatiron EHR], DAB+TRAM and VEM+COBI). An unadjusted comparison of OS across treatment groups will be conducted using Kaplan-Meier (KM) analyses. OS over time in these treatment groups will be plotted and compared using a log-rank test, with median survival time and survival proportions reported at selected time points (e.g., 6 months, 1 year, 2 years).

Hazard ratios for OS comparing ENCO+BINI relative to DAB+TRAM and VEM+COBI will be estimated based on a univariable Cox proportion hazards model. The proportional hazards assumption will be assessed using tests of Schoenfeld residuals.[14]

Imputation of missing data on baseline characteristics

Previous analyses of the Flatiron data have found that there are moderate to large amounts of missing data on baseline characteristics such as ECOG performance score and LDH.[12] *As analyses restricted to patients with non-missing data are highly likely to be biased, imputation of missing data on key baseline characteristics is needed to ensure that adjusted analyses of OS between the trial and RWD settings are based on all eligible patients.*

In this study, multiple imputation by chained equations (MICE)[15] *will be used to impute missing data on baseline characteristics. Briefly, the MICE method imputes data for a missing patient characteristic based on iterative regressions of observed data for that characteristic on observed and imputed values of other baseline characteristics. This process will be repeated for each characteristic until stable estimates are obtained, and the entire procedure will be repeated so that multiple datasets with imputed values for baseline characteristics are created.*

Baseline characteristics to be imputed will include ECOG performance score, LDH, and other clinical or demographic characteristics (e.g., race, BMI, etc.). Additional variables (e.g., age, sex, AJCC diagnosis, time from MM diagnosis to treatment initiation, time from initial diagnosis of melanoma to metastatic disease, number of organs at baseline, metastatic sites, treatments in the therapeutic setting, treatments in the adjuvant setting) will be considered for inclusion in the MICE procedure based on their relevance for adjustment and degree of missingness in the study sample. As recommended in the literature, the MICE approach will be used to create multiple datasets (e.g., 100 datasets) with imputed values, which will be used in subsequent analyses.[15]

Missing data on baseline characteristics will be imputed for all treatment groups. Analyses of baseline characteristics will be repeated for each imputed dataset, and then summarized across all imputed datasets.

Adjusted comparison of OS between treatment groups

Finally, an adjusted comparison of OS across treatment groups (i.e., ENCO+BINI [pooled across COLUMBUS and Flatiron EHR], DAB+TRAM and VEM+COBI) will be conducted. Hazard ratios will be estimated based on a multivariable Cox proportional hazard model, adjusting for key baseline characteristics known or expected to be related to OS in metastatic melanoma (e.g., age, sex, race, BMI, ECOG, LDH, time from initial melanoma diagnosis to metastatic disease, time from metastatic diagnosis to treatment initiation, treatment experience in the therapeutic setting, prior IO/chemotherapy in the adjuvant setting, prior surgery in the adjuvant setting). Specific adjustment factors listed here may be revised based on expert clinical input.

The adjusted analyses will be conducted in each of the datasets with imputed baseline characteristic values in turn. Log-hazard ratios for OS comparing treatment with ENCO+BINI relative to DAB+TRAM and VEM+COBI each imputed dataset will be obtained, and summary HRs obtained by pooling results across all imputed datasets will be generated using Rubin's rules.[16]

Exploratory analyses of PFS

Analyses assessing PFS will also be explored. PFS will be defined as described in Section 8.3. PFS will be summarized for all treatment groups.

Suitability of comparative analyses of PFS between treatment groups will be determined following additional data exploration to characterize number and frequency of assessments of PFS available in these settings, and discussion of comparability of the assessment of disease progression in RWD vs CTD. If analyses of PFS are deemed suitable based on these evaluations, unadjusted and adjusted comparisons of PFS will be conducted using the methods described for OS above.

Sensitivity analyses

Two sets of sensitivity analyses may be pursued. First, Cox regression analyses for OS and PFS may be repeated with adjustment for additional covariates based on clinical input. Second, given differences in timing of approvals of each of the targeted therapies of interest, and changes in the treatment landscape over the time frame, analyses exploring sensitivity of the results to time frames of treatment initiation may also be explored based on clinical input.

7.2 STATISTICAL ANALYSES

Table 3 below outlines the analyses to be conducted in the full analysis set (defined by inclusion and exclusion criteria in Section 4.1).

Table 3 List of analyses to be conducted

Analysis	Endpoint(s)	Statistical method	Covariates	Sensitivity/exploratory analyses	Subgroup analyses
Baseline demographics, clinical characteristics, and LOT-related variables	Categorical variables: sex, race, ECOG at index, AJCC disease stage at initial melanoma diagnosis, AJCC stage at treatment initiation, LDH at index, year of treatment initiation, region, BRAF mutation status, treatment experience in the therapeutic setting, prior systemic therapies in the adjuvant setting, prior IO/chemotherapy in the adjuvant setting, prior surgery in the adjuvant setting,	Frequency counts (n), percentages (%), missing/unknown.	--	None	None

	prior radiotherapy in the adjuvant setting, number of lines of treatment after targeted therapy treatment initiation, number of organs involved, presence of lung metastases, presence of liver metastases, presence of other metastases				
	Continuous variables: age at baseline, BMI at index, time from initial melanoma diagnosis to metastatic disease, time from metastatic melanoma diagnosis to treatment initiation	N/missing; mean/SD; median/IQR	--	None	None

Primary analysis of outcome	OS	ICox proportional hazards models comparing ENCO+BINI to DAB+TRAM and VEM+COBI, adjusted for baseline characteristics	*key baseline characteristics known or expected to be related to OS in metastatic melanoma (e.g., age, sex, race, BMI, ECOG, LDH, time from initial melanoma diagnosis to metastatic disease, time from metastatic diagnosis to treatment initiation, treatment experience in the therapeutic setting, prior IO/chemotherapy in the adjuvant setting, prior surgert in the adjuvant setting). Specific adjustment factors listed here may be*	Analyses may be repeated with adjustment for additional covariates based on clnical input. Given differences in timing of approvals of each of the targeted therapies of interest, and changes in the treatment landscape over the time frame, analyses exploring sensitivity of the results to time frames of treatment initiation may also be explored based on clinical input.	None

			revised based on expert clinical input.		
Exploratory analysis of outcome	PFS	Cox proportional hazards models comparing ENCO+BINI to DAB+TRAM and VEM+COBI, adjusted for baseline characteristics	*key baseline characteristics known or expected to be related to PFS in metastatic melanoma (e.g., age, sex, race, BMI, ECOG, LDH, time from initial melanoma diagnosis to metastatic disease, time from metastatic diagnosis to treatment initiation, treatment experience in the therapeutic setting, prior IO/chemotherapy in the adjuvant setting, prior surgert in the adjuvant setting). Specific*	Analyses may be repeated with adjustment for additional covariates based on clnical input. Given differences in timing of approvals of each of the targeted therapies of interest, and changes in the treatment landscape over the time frame, analyses exploring sensitivity of the results to time frames of treatment initiation may also be explored based on clinical input..	None

			adjustment factors listed here may be revised based on expert clinical input.		

8 LIST OF TABLES

Table	*Description*
Table 1. Key exposures, outcomes, and patient baseline characteristics in the treatment groups	*Table summarizing exposure, outcome, and patient baseline characteristics that will be evaluated within the study.*
Table 2. Kaplan-Meier analyses comparing OS between treatment groups	*Table summarizing KM analyses from the ENCO+BINI, DAB+TRAM and VEM+COBI treatment groups*
Table 3. Unadjusted and adjusted hazard ratios comparing OS between treatment groups	*Table summarizing hazard ratios comparing ENCO+BINI to the DAB+TRAM and VEM+COBI treatment groups*

9 APPENDICES

9.1 APPENDIX 1: TABLE SHELLS

Appendix Table 1: *Baseline patient characteristics, by treatment group*

	ENCO+BINI	*VEM+COBI*	*DAB+TRAM*
	N =	*N =*	*N =*
Demographic Characteristics			
Age at baseline (years)			
Mean (SD)			
Median (IQR)			
Sex, n (%)			
Female			
Male			
Missing, n (%)			
Race, n (%)			
White/Caucasian			
Black/African American			
Asian			
Native American or Pacific Islander			
Other			
Missing			
BMI (kg/m2)			
Mean (SD)			
Median (IQR)			
Missing, n (%)			
Year of treatment initiation, n (%)			
2014			
2015			
2016			
2017			
2018			
2019			
2020			

2021			
Region, n (%)			
North America			
Europe			
Australia			
Other			
Missing			
Disease-Related Characteristics			
Number of organs involved (metastatic sites) at baseline, n (%)			
1			
2			
≥3			
Missing			
Lung metastasis, n (%)			
No			
Yes			
Missing			
Liver metastasis, n (%)			
No			
Yes			
Missing			
Other metastasis, n (%)			
No			
Yes			
Missing			
Time from initial melanoma diagnosis to metastatic disease (months)			
Mean (SD)			
Median (IQR)			
Missing, n (%)			
Time from metastatic melanoma diagnosis to treatment initiation (months)			
Mean (SD)			
Median (IQR)			
Missing, n (%)			
BRAF mutation status, n (%)			
V600E			
V600K			
ECOG performance status, n (%)			
0			
1			
≥2			
Missing			
AJCC stage at initial melanoma diagnosis, n (%)			
0			
I			
II			
III			
IV			
Unknown			
Missing			

AJCC stage at treatment initiation, n (%)			
Stage IIIC			
Stage IV			
Missing			
LDH categories, n (%)			
≤ ULN			
> ULN			
Missing			
Treatment-Related Characteristics			
Treatment experience in the therapeutic setting, n (%)			
Treatment naive			
Prior 1L IO therapy			
Prior systemic therapies in the adjuvant setting, n (%)			
Yes			
No			
Prior IO or chemotherapy in the adjuvant setting, n (%)			
Yes			
No			
Prior surgery in the adjuvant setting, n (%)			
Yes			
No			
Number of LOTs after targeted therapy, n (%)			
0			
1			
≥2			
Missing			

Appendix Table 2: *Kaplan-Meier analyses comparing OS between treatment groups*

	ENCO+BINI	*VEM+COBI*	*DAB+TRAM*
	N =	*N* =	*N* =
Summary of deaths, censoring and median survival			
Death, n (%)			
Censored, n (%)			
Median follow-up (95% CI)			
Median OS (95% CI)			
Kaplan-Meier based estimates of % of patients remaining alive at different time points			
6 Months			
12 Months			
18 Months			
24 Months			
30 Months			
36 Months			

Appendix Table 3: *Unadjusted and adjusted hazard ratios comparing OS between treatment groups*

Results from adjusted models in Appendix Table 3 will be based on multiple imputation via multivariate imputation by chained equations. Expected adjustment factors are listed below; specific functional forms/categorization of adjustment factors will be based on the data.

	Unadjusted model	*Adjusted model*
	HR (95% CI)	*HR (95% CI)*
Treatment group		
DAB+TRAM vs. ENCO+BINI (ref)		
VEM+COBI vs. ENCO+BINI (ref)		
Adjustment factors		
Age (years)		
Male vs female		
Asian race vs. White		
Other race vs. White		
Unknown race vs. White		
BMI (kg/m²)		
ECOG (1 vs. 0)		
LDH (>ULN vs. ≤ULN)		
Treatment naïve vs. prior 1L IO treatment		
Prior IO/chemotherapy (Yes vs. No)		
Prior surgery (Yes vs. No)		
Time from initial melanoma diagnosis to metastatic disease		
Time from metastatic disease to treatment initiation		

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