NCT03929029

Neoantigen Vaccine Plus Locally Administered Ipilimumab and Systemic Nivolumab in Advanced Melanoma

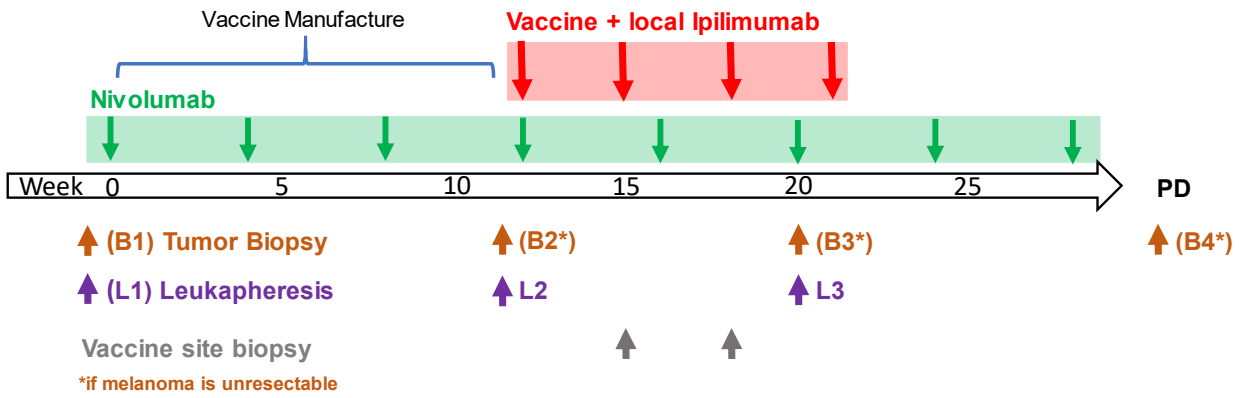
DFCI Protocol #: 18-279

Statistical Analysis Plan

November 20, 2023

Design: The study is an open label, phase Ib trial in which patients with stage III B/C/D or stage IV melanoma were treated with NeoVax plus Montanide® ISA-51 VG in combination with nivolumab and locally administered ipilimumab. Tumor from a surgical resection specimen or core needle biopsy of a melanoma metastasis was used to prepare DNA and RNA for sequencing. Patients began treatment with nivolumab, and vaccine was prepared during the initial 12 weeks of nivolumab therapy. At week 12, patients began vaccination with up to 20 neoantigen peptides. These peptides were encoded by non-silent mutations that were identified through DNA and RNA sequencing. Up to 20 peptides of approximately 20 amino acids in length were prepared for each patient and were administered together with the immune adjuvant poly-ICLC and Montanide® ISA-51 VG, a mineral oil-based immune adjuvant analogous to incomplete Freund's adjuvant (IFA).

Concurrently with the vaccine and nivolumab, ipilimumab was delivered via subcutaneous injection in proximity to each vaccination site to 1) direct anti-CTLA-4 activity to the vaccine-draining lymph nodes, and 2) limit systemic toxic effects. This approach has been shown to be effective in animal tumor models and was expected to result in significantly reduced levels of systemic ipilimumab compared to the approved dose/schedule in advanced melanoma (3 mg/kg q3wks for four doses in the metastatic setting). The ipilimumab dose would be escalated/de-escalated from 2.5 mg/kg flat dose per injection in Cohort 1. Serial tumor biopsies and vaccine site biopsies were performed as shown in the schema below.



The primary endpoint is the rate of DLTs (CTCAE V5.0), as outlined and defined in Section 5.5 of the protocol. We chose dose escalation cohorts of five patients, rather than a more traditional 3+3 design, because the determination of whether to treat at a different dose in this trial was made over a longer period due to the time needed for vaccine preparation and the extended period for safety evaluation. Three dosing cohorts were considered, each varying the dose of ipilimumab. The decision rule to escalate to the next dose was based on having zero or one patient (out of five) experiencing a DLT during the first 42 days of vaccine therapy. The first cohort, Cohort 1, was treated with Ipilimumab 2.5 mg per injection site.

If zero or 1 patient in Cohort 1 experienced a dose limiting toxicity (DLT) during the first 7 weeks of treatment, 5 patients would be treated as Cohort 2 (Ipilimumab 5 mg per injection site). If two or more patients in Cohort 1 experienced a DLT during the first 7 weeks of treatment, then 5 patients would be treated at Cohort -1 (Ipilimumab 1.25 mg per injection site). If none or 1 patient experienced a DLT on Cohort 2, then Ipilimumab at 5 mg per injection site would be the maximum tolerated dose (MTD) and an additional 10 patients will be treated at that dose level to increase the likelihood of detecting serious

toxicities, to complete biologic correlative endpoints, and to gain preliminary experience with clinical tumor activity. If two or more patients in Cohort 2 experienced DLT, then Dose Level 1 would be the MTD and an additional 10 patients would be treated in an expansion cohort at this dose.

If no or only 1 patient experienced a DLT on Cohort -1, then Ipilimumab 1.25 mg per injection site would be the MTD and an additional 10 patients would be treated in an expansion cohort at that dose level. If two or more patients in Cohort -1 experienced DLT, then the study will be stopped and no MTD would be declared.

The table below shows the probability of moving to the dose expansion phase (0 or 1 patients with DLT out of 5 patients at the MTD) for various possible values of the true, but unknown, toxicity rate.

True rate of DLT (%)	Probability of Dose Expansion (%)
10	92
20	74
30	53
40	34
50	19
60	9

If the true probability of DLT is 10% or less, then the probability of proceeding to dose expansion is at least 92%. The probability of dose expansion is less than 50% if the true rate of dose-limiting toxicities is 32% or greater.

Minimum vaccination requirement to pass DLT observation period: A minimum of 2 vaccinations and absence of DLTs are required for a patient to complete the 7-week DLT observation period successfully.

Modified Design: Due to slow accrual in this trial, enrollment to the expansion cohort did not begin and trial accrual was stopped at the end of the dose escalation phase, at the time the MTD was determined.

Endpoints

1. Primary Endpoint: The primary endpoint was safety and toxicity, specifically the rate of DLT. Adverse events to assess the safety and side-effect profile of the NeoVax were recorded using Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

2. Secondary Endpoints

a. Objective antitumor response: Best overall response (complete response (CR) or partial response (PR)) per RECIST 1.1.

b. Disease progression/recurrence: The number of patients with disease progression among those with unresectable disease, or disease recurrence in those with resectable disease.

Variables and Derived Data

1. Patient Enrollment and Demographics

CRF: q_demo

Variable Name	Values
Casenum	Patient ID
Cohort	Cohort designation
Dt_regdt	Study registration date
Dt_birthdt	Date of birth
D_sex	Sex
D_race	Race
D_ethnic	Ethnicity

Code for age (years):

age = floor((dt_regdt - dt_birthdt)/365.25);

Code for cohort:

If 101 le casenum le 106 then cohort = “Cohort 1”;
else cohort = “Cohort 2”;

Note: Case #102 did not receive NeoVax due to insufficient neoantigens for vaccine preparation.

2. Disease Characteristics

CRF: q_studyentry

Variable Name	Values
d_stagemelanoma	Melanoma AJCC stage

3. Response to Therapy

CRF: q_resp

Variable Name	Values
D_statover	Best response to therapy
DT_DTdisassess	Date best overall response
D_conf	Was response confirmed?

4. On-study Toxicities

CRF: q_ae

Variable Name	Values
C_st	Flag for timing of AE (remove 0=baseline)
toxcode	CTCAE toxicity code
C_toxgrd	Toxicity grade
c_attrib	Relatedness to NeoVax (if 2, 3, 4 then deemed at least possibly related)

c_attribnivo	Relatedness to Nivolumab (if 2, 3, 4 then deemed at least possibly related)
c_attribipi	Relatedness to Ipilimumab (if 2, 3, 4 then deemed at least possibly related)
toxdesc	Text for toxicity name
toxcat	MEDRA System Organ Class
D_toxdlt	Was event a DLT?
COMM250	Description text for toxicities coded as “other”

Toxicities presented according to worst grade occurring per patient:
Proc sort data=tox; by subject toxcode descending c_toxgrd;

```
data tox_unique;
set tox; by casenum toxcode;
if first.toxcode;
if index(toxdesc,"Other") gt 0 then toxdesc = propcase(COMM250);
run;

proc tabulate data=tox_unique format=6.1;
class toxcode toxdesc toxcat c_toxgrd/style={font_weight=bold};
table toxcat="System-Organ Class"*toxdesc = "Description",(all="Number patients"
c_toxgrd="Grade")*N/misstext="-";
run;
```

To select toxicities that are at least possibly related to therapy:
Subset to those events where c_attrib* is 2, 3, or 4.

5. Deaths and Overall Survival

CRF: q_survival

Variable Name	Values
DT_DTDEATH	Month/day/year death
DT_DTCONT	Month/day/year last follow-up
C_STATUS	Patient status (death = 2)

6. Time to Progression

CRF: q_resp

Variable Name	Values
C_STATOVER	Value = 5 for patients with PD
DT_DTPROG	Date of disease progression

Statistical Analyses

Primary Endpoints

Safety and toxicity

a. DLT Rate

Proportion of patients who developed a DLT as a result of NeoVax therapy.

Secondary Endpoints

a. Best Response to Therapy

The number of patients with best response of CR or PR (per RECIST 1.1) will be summarized. For rate estimation, the denominator is the number of patients in each cohort.

b. Disease Progression/Recurrence

The number of patients with disease progression (unresectable) or disease recurrence (resectable).