Patients with high-risk melanoma have a 20-60% recurrence rate with 5-year OS between 45% and 70%. The adjuvant setting is an opportunity to test prevention vaccines that may have efficacy against disease recurrence.

We evaluated vaccination with CDX-1401, a fusion protein consisting of human monoclonal IgG1 antibody targeting the dendritic cell (DC) receptor DEC-205 linked to the full-length NY-ESO-1 tumor antigen, with or without pretreatment with CDX-301, a recombinant human Flt3 ligand (RFlt3L), in a phase II, open-label, multicenter, randomized study of subjects with resected Stage IIIB-IV melanoma.

**Primary Objective:**
- To determine whether the immune response to NY-ESO-1 elicited by vaccination with CDX-1401 and Hiltonito® (Poly-ICLC, from Oncovir) is substantially increased by prior expansion in number of circulating dendritic cells (DC) by therapy with CDX-301.

**Secondary Objectives:**
- To assess the effect of the vaccine regimen on immune responses to other ongoing and nascent antitumor response antigens associated with melanoma.
- To assess the effect of the vaccine regimen on the frequency and phenotypic character of peripheral blood mononuclear cell (PBMC) subsets including DCs, monocyte populations, T cells, and natural killer (NK) cells.
- To assess the safety, tolerability, and clinical efficacy of the vaccine regimens

**Methods**

**Trial Design:** 60 pts with resected melanoma were randomized to two cohorts:
- Cohort 1 received CDX-301 pretreatment (25 mg/kg SC daily x 10 days) in 2 of 4 monthly cycles of vaccination with CDX-1401 (1 mg IC day 1) + poly-ICLC (2 mg SC, days 1 and 2).
- Cohort 2 received 4 monthly cycles of vaccine with CDX-1401 and poly-ICLC w/o prior CDX-301.

**ELISpot:** Antigen-specific IFNγ release was determined using a direct 48 hour ELISpot assay using thawed and overnight-rested PBMCs (100,000/well) exposed to NYESO-1 peptide pools (15mers, overlapping by 11 aa).

**Flow Cytometry:** Whole Blood was stained with a 12-color flow cytometry panels designed to measure changes in absolute cell numbers of the major PBMC subsets or T cell subsets.

**Gene Expression:** Rins from PBMC was prepared in RNAlater and analyzed for gene expression levels using the Nanostring 770 gene nCounter® PanCancer Immune Profiling Panel.

**Results**

- The combination regimens of Flt3L (CDX-301), DC targeted NY-ESO-1 (CDX-1401) and poly-ICLC were well tolerated.
- Substantial increases in innate immune cells (dendritic cells, NK cells and Monocytes) were elicited in Flt3L treated patients (CH1).
- A significant increase in antibody titer was noted in both cohorts, but the average peak titer was higher in CH1 vs CH2.
- CH1 demonstrated greater increases in NY-ESO-1 specific T cell responses compared to CH2. Moreover, the response was induced after only one injection in CH1 whereas, two injections were required in CH2.

**Conclusions**

- The combination regimens of Flt3L (CDX-301), DC targeted NY-ESO-1 (CDX-1401) and poly-ICLC were well tolerated.
- Substantial increases in innate immune cells (dendritic cells, NK cells, and monocytes) were elicited in Flt3L treated patients (CH1).
- Antibody titers were elicited in both cohorts, but the average peak Ab titer was significantly higher in CH1 vs CH2.
- Antigen-specific (anti-NY-ESO-1) T cell responses were elicited in both cohorts, however:
  - The number of responders in CH1 (Flt3L treated) was statistically greater vs CH2.
  - Responses in CH2 were detectable earlier and were more robust.
- Little/no evidence of ‘epitope spreading’ was detected in either cohort.
- DC mobilization with CDX-301 is safe and may enhance responses to DC targeted vaccines.
- Additional correlation and GEP (Gene Expression Profiling) studies are in progress.