A Phase 1b Study of OrienX010, an Oncolytic Virus, in Unresectable Stage IIIC to IV Melanoma Patients

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SUPPLEMENTARY FILE

Supplement Table 1. Main inclusion and exclusion criteria

Main inclusion criteria:

Patients are eligible to be included in the trial only if the following criteria apply:

1. Male or female ≥ 18 years old on day of signing informed consent;
2. Histologically and/or cytologically confirmed Stage IIIb, IIIc or IVM1a malignant melanoma, which has progressed or recurred on conventional therapies, or for which there is no available effective treatment available;
3. ECOG performance status of 0-2;
4. Minimum life expectancy of 3 months;
5. Prior anti-tumor treatments (including chemotherapy/radiotherapy, immunotherapy, targeted therapy, endocrine therapy or others) completed at least 4 weeks prior to study enrollment (at least 6 weeks if nitrosoureas and mitomycin chemotherapy were used);
6. At least 1 measurable lesion (diameter ≥ 10 mm) and suitable for intratumoral injection;
7. Adequate organ function as defined below at the time of screening:

a. White blood cell count ≥ 3.0×10^9/L; absolute neutrophil count ≥ 1.5×10^9/L; platelet count ≥ 75×10^9/L;
b. Serum creatinine ≤ 1.5 times ULN;
c. Total bilirubin ≤ 2.0 times ULN;
d. ALT and AST ≤ 2.5 times ULN;
e. INR ≤ 2.0;

8. Provide signed informed consent with an expectation of good compliance with study procedures

Main exclusion criteria:

Patients are excluded from the trial if any of the following criteria apply:

1. Is currently participating in another clinical trial and has received interventional therapies within 4 weeks prior to the first dose of study treatment; exception: prior enrollment in a non-interventional study (eg, epidemiologic study) is allowed;
2. Received anti-HSV therapy (such as acyclovir, ganciclovir, valacyclovir or vidarabine) within 4 weeks prior to the first dose of study treatment;
3. Major surgical procedure(s) within 4 weeks prior to the first dose of the study treatment;
4. History of other (including unknown primary) malignancies within 5 years prior to the first dose of trial treatment, with the exception of skin cancers other than malignant melanoma, breast carcinoma in situ, and cervical carcinoma in situ;
5. History or current evidence of severe diseases, including but not limited to: severe heart disease, cerebrovascular disease, uncontrolled diabetes, uncontrolled hypertension, serious infection, active peptic ulcer, severe immune dysfunction, etc., which in the opinion of the treating investigator, may jeopardize the patient's safety or affect the completion of the study;
6. Uncontrolled mental illness, infectious disease, or skin disease;

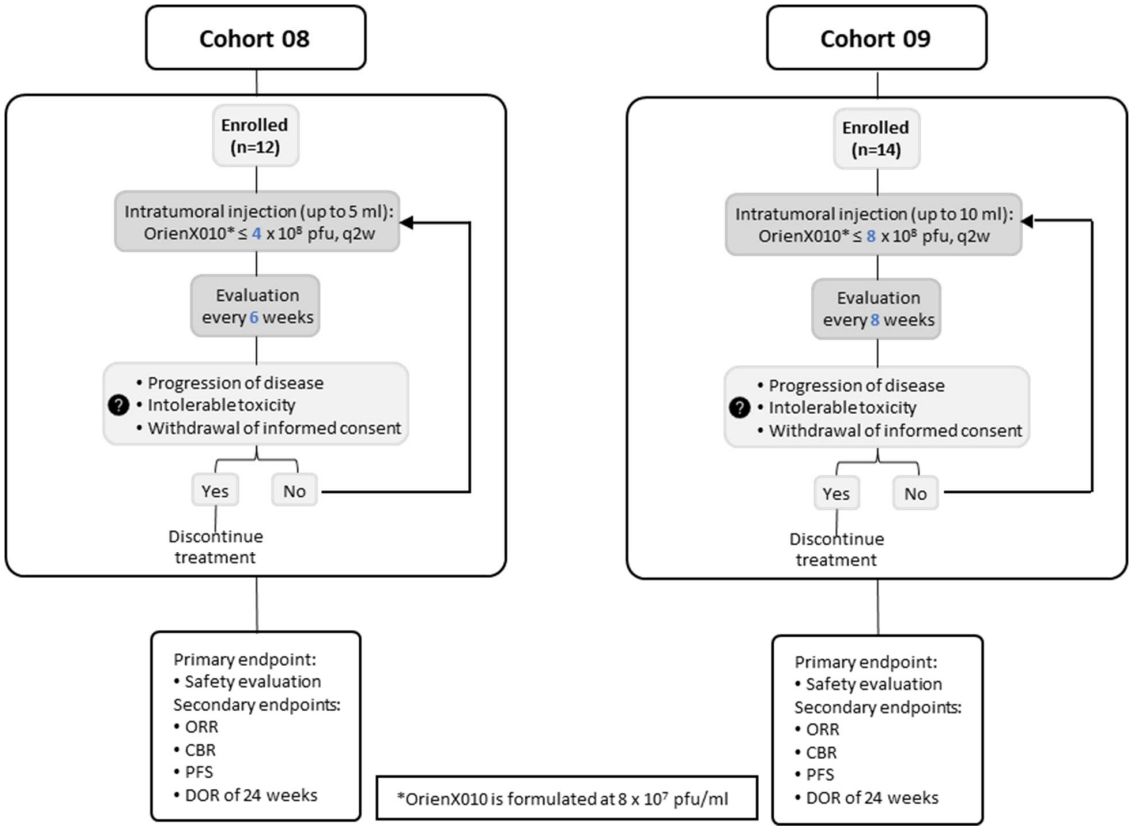
7. Lesions that do not meet the requirement of the intratumoral injection volume or is not suitable for intratumoral injection; 8. Large lesion (diameter > 100 mm); 9. Negative anti-HSV-1 antibodies IgG and IgM; 10. Known hypersensitivity to the study drug, its active ingredient, or its excipients; 11. Pregnant or lactating women; 12. Childbearing woman or spouse of childbearing woman and not using a medically acceptable contraception method (hormonal or shielding or abstinence) during the study; 13. Other situations unsuitable for enrollment according to the opinion of the treating investigator
ALT, alanine aminotransaminase; AST, aspartate aminotransferase; ECOG, Eastern Cooperative Oncology Group; HSV, Herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; INR, international normalized ratio; ULN, upper limit of normal

Supplement Table 2. Dosing: Relationship of lesion size and injectable volume

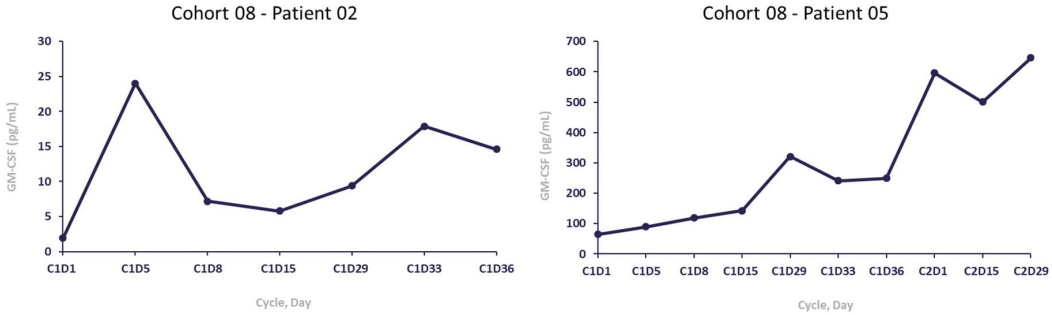
Diameters (cm)	Injectable volume of OrienX010 (mL)[a]
> 7 and ≤ 10	10
> 5.5 and ≤ 7	8
> 3.5 and ≤ 5.5	6
> 2.5 and ≤ 3.5	4
> 1.5 and ≤ 2.5	2.5
≤ 1.5	≤ 1.25
[a]Maximal dosing volume could not exceed 10 mL (maximal dose: 8×10^7 pfu/mL x 10 mL = 8×10^8 pfu)	

Supplement Table 3. Specimen collection timepoints

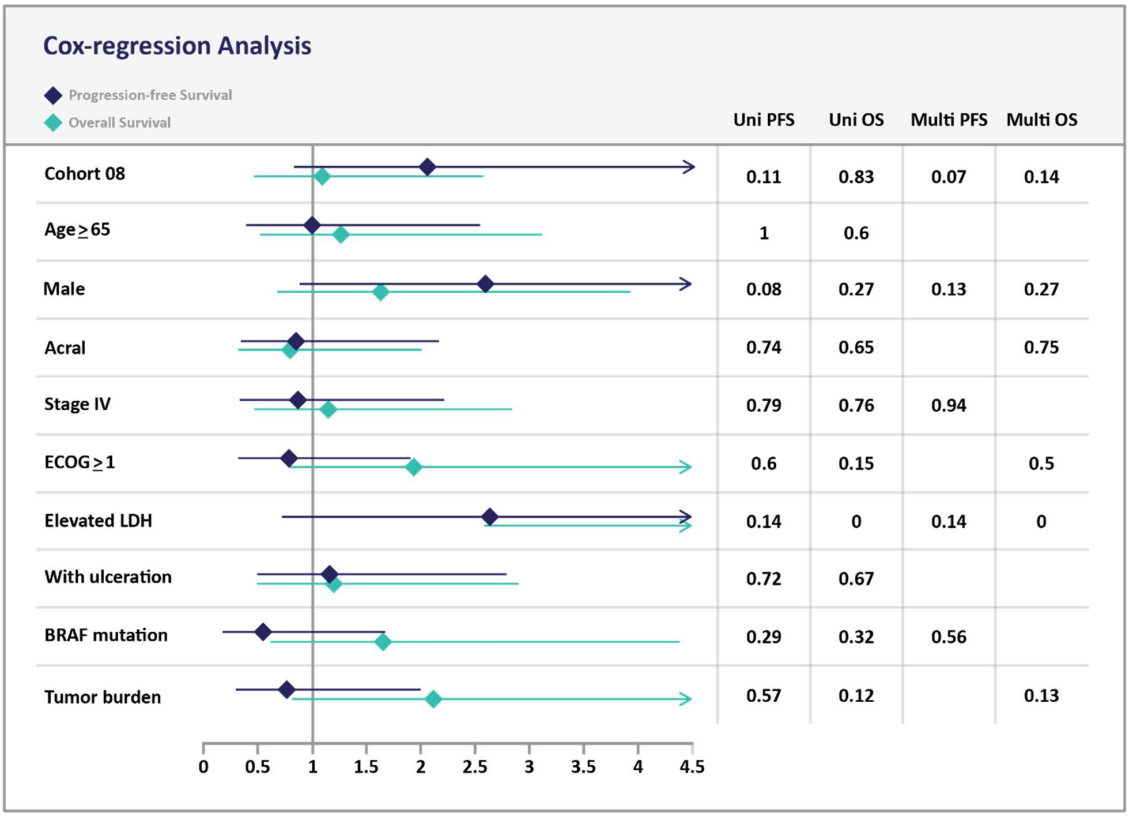
Cotton swab of injection site	Cohort 08: 24 hours post dose on C1D1, C1D15, C1D29, C1D43, C2D1, C2D15, C2D29 and C2D43	Cohort 09: 24 hours post dose on C1D1, C1D15, C1D29, C1D56, C2D1, C2D15, C2D29 and C2D56
Serum and urine		
C1D1:	Pre-dose, 4 hours, 8 hours, 24 hours, 48 hours post dose	
C1D15:	Pre-dose	
C1D29:	Pre-dose	
C1D43:	Pre-dose, 4 hours, 8 hours, 24 hours, 48 hours post dose	
C2D1:	Pre-dose	
C2D15:	Pre-dose	
C2D29:	Pre-dose	
C2D43:	Pre-dose	
Serum (ADA)	Pre-dose on C1D1, C1D15, C1D29, C1D43, C2D1, C2D15, C2D29 and C2D43	
Core needle biopsy	Baseline (C1D1) and at 12 weeks (C3D1)	Baseline (C1D1) and at 16 weeks (C3D1)
ADA, anti-drug antibodies; C, cycle; D, day.		



Supplement Figure 1. Study schema of cohorts 08 and 09. CBR, clinical benefit rate; DOR, duration of response; ORR, objective response rate; PFS, progression-free survival; q2w, every 2 weeks.



Supplement Figure 2. **GM-CSF induction.** The concentration of GM-CSF in the blood was evaluated using ELISA at multiple time points prior to injection of OrienX010. Of the 26 patients in cohort 08 and cohort 09, 2 patients in cohort 08 were found to have substantial increase in GM-CSF. Patient 02 (**left**) had a 12.6-fold increase, and patient 05 (**right**) a 10.0-fold increase in GM-CSF compared to pre-treatment baseline levels on C1D1. C, Cycle; D, Day; ELISA, enzyme-linked immunosorbent assay; GM-CSF, granulocyte-macrophage colony-stimulating factor.



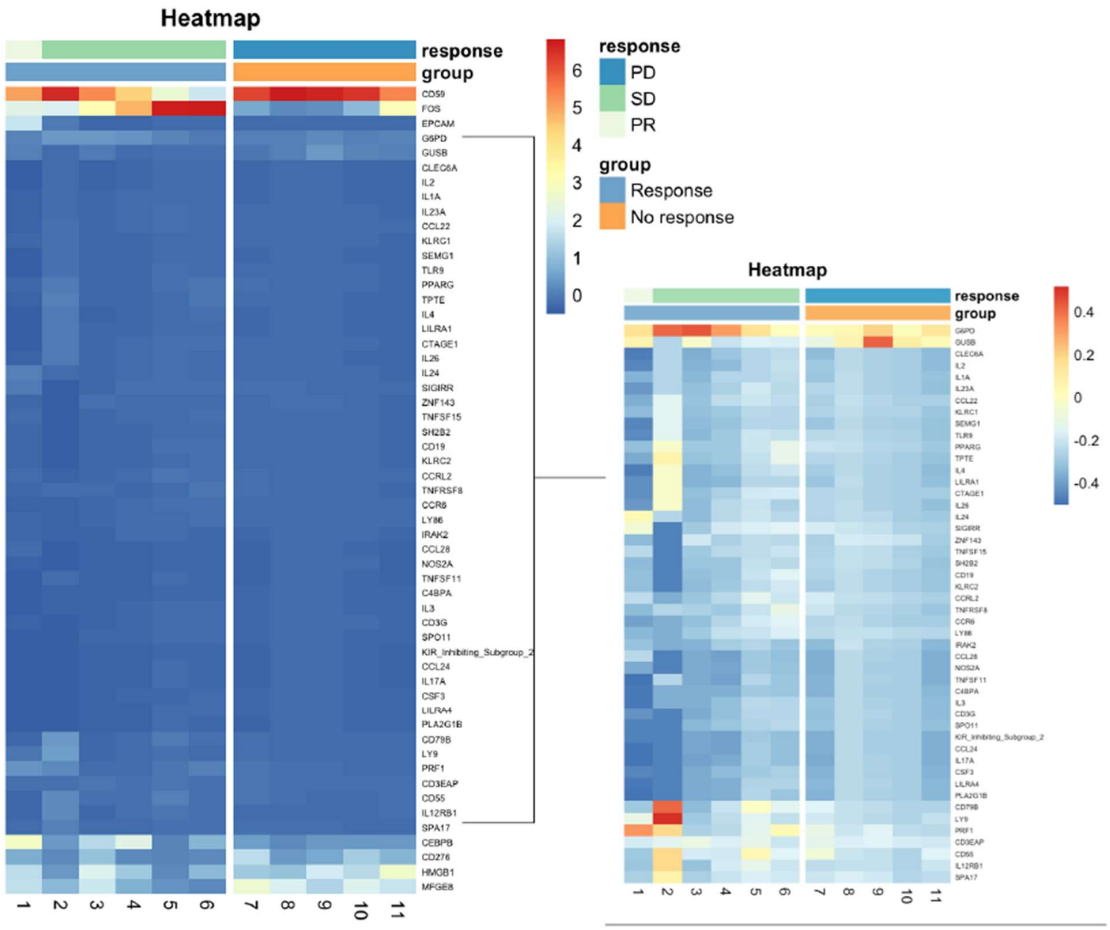
Supplement Figure 3. Univariate and multivariate analysis of all 26 patients who received OrienX010. The line in the middle shows the hazard ratio and 95% CI of each factor. Uni PFS (OS) is the p-value of each factor in the univariate analysis of PFS (OS). Multi PFS (OS) is the p-value of different factors included in the multivariate analysis of PFS (OS). ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival.

NanoString analysis

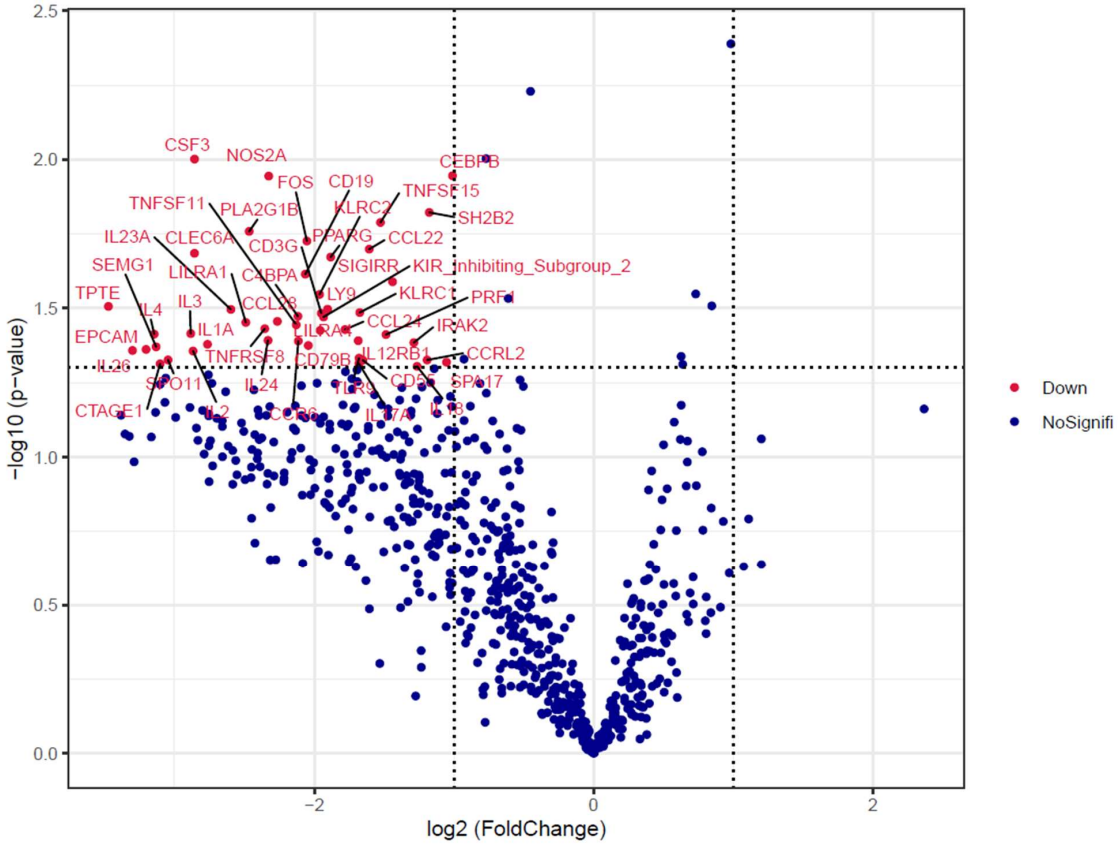
To identify potential mechanisms of therapeutic resistance, a targeted gene expression profile (GEP) was performed via an nCounter® PanCancer Immune Profiling Panel in tumor samples with available tissue. In total, 11 samples were taken from 4 patients in cohort 08 and 7 patients in cohort 09. Among these 11 patients, 1 achieved partial response (PR) and 5 achieved stable disease (SD) as best responses. The other 6 experienced progressive disease (PD) during OrienX010 treatment. When comparing the pre-treatment GEP results between patients with PR or SD and those with PD, 55 genes were significantly differentially expressed ($p \leq 0.05$), 46 of which were highly expressed (defined as a difference of more than 2 fold) in the 6 patients achieving PR and SD (Supplement Figures 4 and 5).

These 46 genes were analyzed using Metascape and the top 20 enriched clusters are shown in Supplement Figure 6, most of which are associated with inflammatory and immune responses. Using Gene Set Enrichment Analysis (GSEA), only 1 pathway (*IL2_STAT5* signaling pathway) was found to be enriched in the response group (eg, with the absolute value of normalized enrichment score ≥ 1, normal p-value ≤ 0.01, false discovery rate [FDR] q-value ≤ 0.25; Supplement Figure 6).

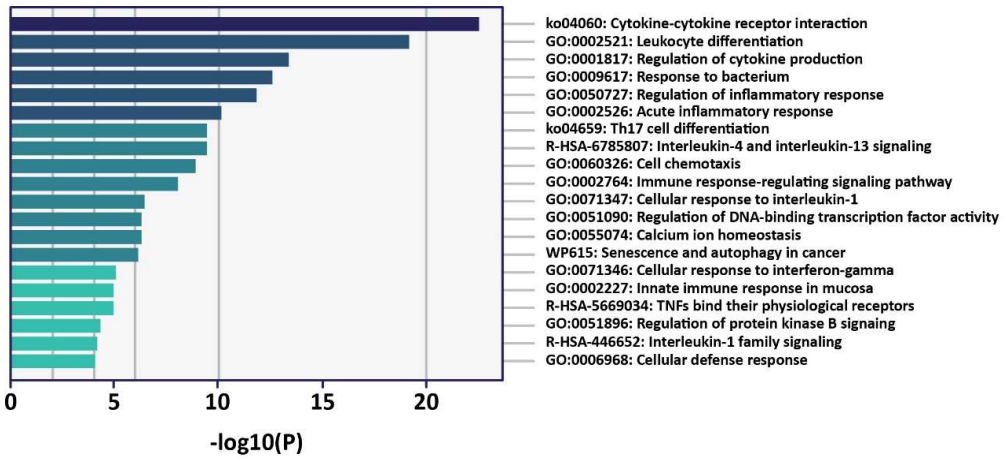
These 46 genes were analyzed using Metascape and the top 20 enriched clusters are shown in Supplement Figure 7, most of which are associated with inflammatory and immune responses. Using GSEA, only 1 pathway (*IL2_STAT5* signaling pathway) was found to be enriched in the response group (eg, with the absolute value of normalized enrichment score ≥ 1, normal p-value ≤ 0.01, false discovery rate [FDR] q-value ≤ 0.25; Supplement Figure 7).



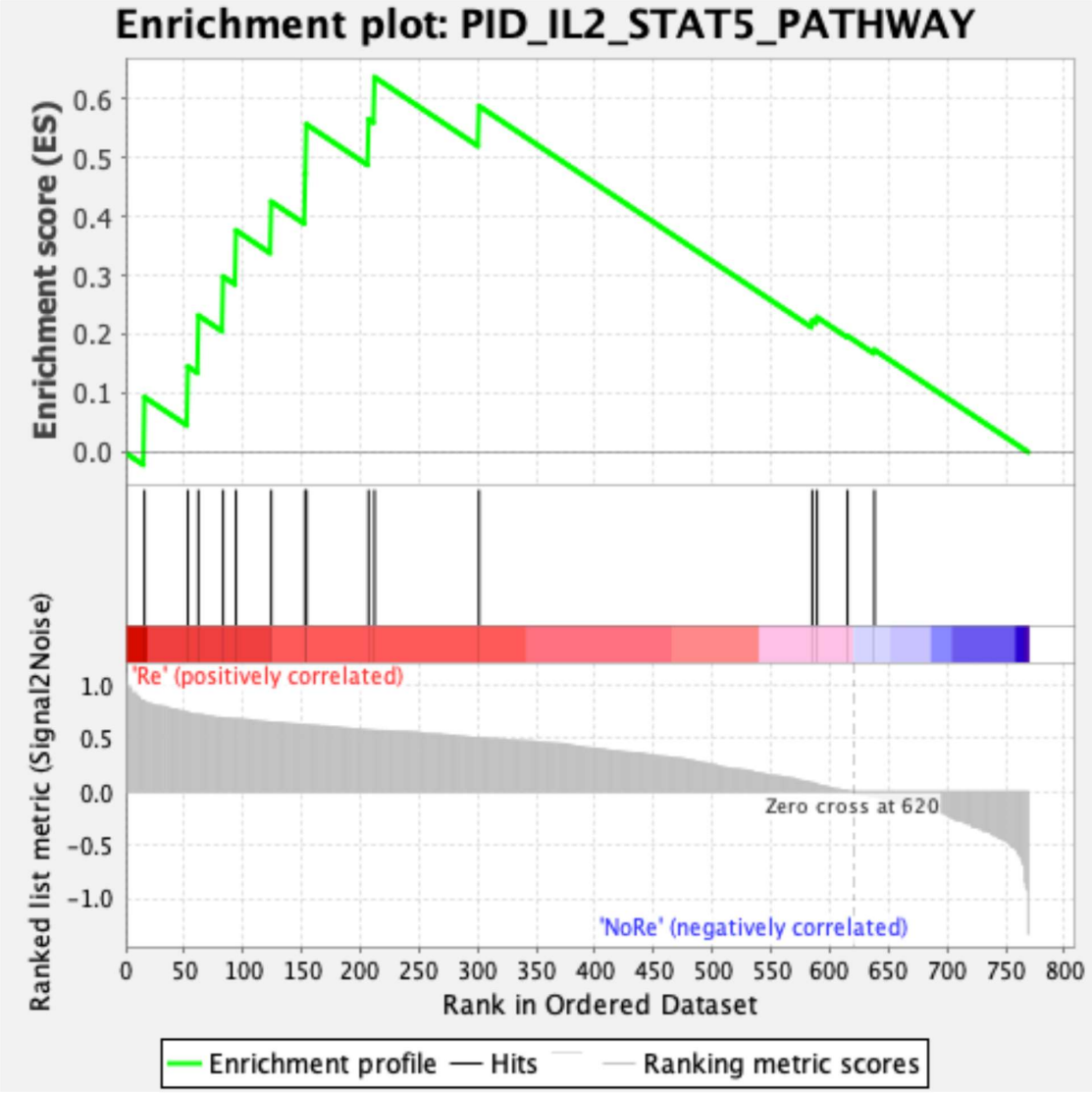
Supplement Figure 4. Heatmap of the 46 highly expressed genes in responders (left); and the differences more clearly shown (right). PD, progressive disease; PR, partial response; SD, stable disease.



Supplement Figure 5. Volcano plot, which shows the differential genes between responders and non-responders. The red dots represent the 46 high-expressed genes in responders.



Supplement Figure 6. Enriched clusters of the 46 high-expressed genes. Th17, T helper 17 cell; TNF, tumor necrosis factor.



Supplement Figure 7. Enriched plots of the enriched pathway in the response group.