|  |  |  |
| --- | --- | --- |
| GENE SYMBOL | mRNA | TESTING RANGE |
| PRF1 | NM\_005041 | Exon2-3 |
| UNC13D | NM\_199242 | whole exon |
| STX11 | NM\_003764 | Exon2 |
| STXBP2 | NM\_006949 | whole exon |
| XIAP | NM\_001167 | Exon2-7 |
| SH2D1A | NM\_002351 | whole exon |
| RAB27A | NM\_004580 | Exon2-7 |
| MYO5A | NM\_000259 | whole exon |
| AP3B1 | NM\_003664 | whole exon |
| LYST | NM\_000081 | Exon3-53 |
| ITK | NM\_005546 | whole exon |
| SLC7A7 | NM\_001126105 | Exon3-9 |
| BLOC1S6 | NM\_012388 | whole exon |
| CD27 | NM\_001242 | whole exon |
| MAGT1 | NM\_032121 | whole exon |
| ADA | NM\_000022 | whole exon |
| BTK | NM\_000061 | Exon2-19 |
| IL2RA | NM\_000417 | whole exon |
| IL2RG | NM\_000206 | whole exon |
| MVK | NM\_000431 | Exon2-11 |
| PNP | NM\_000270 | whole exon |
| WAS | NM\_000377 | whole exon |
| FAS | NM\_000043 | whole exon |
| RECQL4 | NM\_004260 | whole exon |
| RAG1 | NM\_000448 | Exon2 |
| RAG2 | NM\_000536 | Exon2 |

**Supplemental Table 1. The HLH-related gene panel used for HLH diagnosis by next generation sequencing.**

|  |  |  |  |
| --- | --- | --- | --- |
| *Cases* | Primary Treatment | | |
| **CTx (cycles)** | **Treated time (weeks)** | **Outcomes** |
| 1 | ED (1), GED+L-Asp (1), GED (2) | 12 | PR, Relapse |
| 2 | ED (1), GED (2) | 8.4 | NR |
| 3 | ED (1) | 11.3 | PR, Relapse |
| 4 | ED (2) | 10.6 | PR, Relapse |
| 5 | GED (2) | 6.3 | PR, Relapse |
| 6 | ED+CsA (2), GED+CsA (4) | 72 | Relapse |
| 7 | ED (1), GED (1) | 11.1 | NR |

**Supplemental Table 2. Primary chemotherapies and outcomes of seven r/r EBV-HLH patients.**

Abbr: ED, Etoposide + Dexamethasone; GED, Gemcitabine + Etoposide + Dexamethasone; CsA, Cyclosporin A; L-Asp, L-Asparaginase; PR, partial response; NR, no response.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *Case* | *Nivolumab Treatment* | | | | | *Outcomes* | |
| ***Induction*** | | ***Maintenance*** | | | ***Survival (months)*** | ***RFS***  ***(months)*** |
| ***Dose (cycles)*** | ***Outcomes*** | ***Dose (cycles)*** | | ***Outcomes*** |
| 1 | 100mg (1), 200mg (2) | cCR | 140mg (1) 100mg (1) | | cCR | 18.9+ | 18+ |
| 2 | 100mg (6) | cCR and mCR | 100mg (4) | | cCR and mCR | 17.9+ | 17.4+ |
| 3 | 100mg (3) | cCR and mCR | 100mg (2) | | cCR and mCR | 15.3+ | 15.3+ |
| 4 | 100mg (2), 120mg (2), 140mg (2) | cCR and mCR | 100mg (4) | | cCR and mCR | 15.3+ | 13.4+ |
| 5 | 140mg (2), 160mg (1), 180mg (1) | NR, DOD | - | - | | 5 | 0 |
| 6 | 160mg (3) | PR, Relapse | - | | - | 8 | 1.5 | |
| 7 | 100mg (3) | cCR and mCR | 100mg (2) | | cCR and mCR | 11.4+ | 11+ | |

**Supplemental Table 3. Treatments and outcomes of nivolumab infusions.**

Abbr: cCR, clinical complete response; mCR, molecular complete response; PR, partial response; NR, no response; DOD, dead of disease; RFS, relapse-free survival.



**Supplemental Figure 1. Dynamics of clinical and laboratory parameters after nivolumab infusions in two patients without clinical complete response.**

Dynamics of body temperature and plasma EBV-DNA copy numbers in NR patients 6 (A) and patient 5 (B) during induction therapy.



**Supplemental Figure 2. Single cell RNA sequencing analysis of peripheral blood cells of patient 7 before and during nivolumab treatment.**

(A) t-distributed stochastic neighbor embedding (t-SNE) maps displaying peripheral blood cell subpopulations at T0 (Day 0, before infusion), T1 (Day 7 after the first infusion), T2 (Day 21 when clinical CR) and T3 (Day 76 when molecular CR). (B) Violin plots showing the expression levels of marker genes in each cell population at different time points.



**Supplemental Figure 3. Expression levels of TNF, IL1B and CD163 in monocytes/MΦ in peripharal blood of patient 7 before and during nivolumab treatment.** Upper, violin plots showing the expression levels of TNF, IL1B and CD163 in monocytes/MΦ at different time points. Lower, pie charts showing the ratios of TNF, IL1B and CD163 positive monocytes/MΦ at different time points.



**Supplemental Figure 4.** **Expression levels of MKI67 and LAG3 projected on t-SNE plots in the peripheral blood of patient 7 before and during nivolumab treatment.**