**Appendix Figures AND TABLES**

**Figure S1: Phase I study schema of JS001 in advanced melanoma, renal cell carcinoma and urothelial carcinoma.** MEL, melanoma. RCC, renal cell carcinoma. UC, urothelial carcinoma.CR, complete response. PR, partial response. SD, stable disease. PD, progressive disease.

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**Figure S2: The PK profiles of JS001 in humans.** **(A)** PK parameters of JS001 after a single dose for 28 days and multi-dose infusions every two weeks in three dose cohorts of 1 mg/kg, 3 mg/kg and 10 mg/kg. **(B)** Serum concentration of JS001 in three cohorts over 98 days.

| PK Parameter | Unit | 1 mg/kg |  | 3 mg/kg |  | 10 mg/kg |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| JS001 |  | JS001 |  | JS001 |  |
| Mean ± SD | n | Mean ± SD | n | Mean ± SD | n |
| Day29 |
| Kel | 1/hr | 0.005±0.001 | 3 | 0.004±0.002 | 3 | 0.003±0.002 | 3 |
| t1/2 | hr | 149.56±30.93 | 3 | 185.05±84.91 | 3 | 235.68±98.52 | 3 |
| Tmax | hr | 0.5-6.0 | 3 | 0.5-2.0 | 3 | 0.5-2.0 | 3 |
| Cmax | μg/mL | 21.85±5.12 | 3 | 85.36±36.09 | 3 | 232.00±45.22 | 3 |
| AUC(0-t) | hr\*μg/mL | 3263.11±320.28 | 3 | 8841.86±3671.44 | 3 | 36185.78±17056.22 | 3 |
| AUC(0-inf) | hr\*μg/mL | 4143.19±154.40 | 3 | 13307.31±7564.85 | 3 | 65863.66±41568.62 | 3 |
| AUC(t-inf)% | % | 21.16±8.59 | 3 | 27.92±14.91 | 3 | 37.09±20.00 | 3 |
| Vd | mL/kg | 52.08±10.84 | 3 | 68.35±27.93 | 3 | 60.70±21.63 | 3 |
| Cl | mL/hr/kg | 0.24±0.01 | 3 | 0.30±0.22 | 3 | 0.24±0.21 | 3 |
| MRTinf | hr | 215.59±47.06 | 3 | 264.74±124.93 | 3 | 337.61±151.10 | 3 |
| Day113 |
| Kel | 1/hr | 0.003±0.001 | 3 | 0.002±0.001 | 2 | 0.002±0.001 | 2 |
| t1/2 | hr | 228.83±47.43 | 3 | 395.31±186.20 | 2 | 334.11±131.75 | 2 |
| Tmax | hr | 0.5-6.0 | 3 | 0.5 | 2 | 0.0 | 2 |
| Cmax | μg/mL | 47.05±16.97 | 3 | 105.19±32.67 | 2 | 323.77±41.96 | 2 |
| AUC(0-t) | hr\*μg/mL | 5683.85±1285.99 | 3 | 19789.32±3974.65 | 2 | 56328.37±16800.53 | 2 |
| AUC(0-inf) | hr\*μg/mL | 8815.79±2382.45 | 3 | 44022.34±6105.77 | 2 | 126194.03±72330.76 | 2 |
| AUC(t-inf)% | % | 34.44±9.16 | 3 | 53.98±15.41 | 2 | 51.16±14.68 | 2 |
| Cmin | μg/ml | 11.02±1.98 | 3 | 39.64±7.54 | 2 | 127.41±55.69 | 2 |
| Cavg | μg/ml | 16.89±3.82 | 3 | 61.81±11.98 | 2 | 176.36±54.16 | 2 |
| Fluctuation% | % | 206.20±57.58 | 3 | 104.08±20.47 | 2 | 118.10±44.06 | 2 |
| CLss | ml/hr/kg | 0.18±0.04 | 3 | 0.15±0.03 | 2 | 0.18±0.05 | 2 |
| MRTinf | hr | 314.81±80.95 | 3 | 541.63±247.74 | 2 | 491.48±210.36 | 2 |
| Vz | ml/kg | 59.36±14.51 | 3 | 87.80±55.83 | 2 | 80.20±7.45 | 2 |
| Vss | ml/kg | 56.74±17.25 | 3 | 83.28±51.94 | 2 | 81.32±10.52 | 2 |
| Accumulation\_Index |  | 1.57±0.19 | 3 | 2.25±0.77 | 2 | 2.00±0.54 | 2 |
| AUC\_TAU | hr\*μg/ml | 5675.70±1284.05 | 3 | 20766.78±4026.93 | 2 | 59258.36±18198.68 | 2 |

(A)

(B)

**Figure S3: The percentage of activated CD8+ T cell population during JS001 treatment.** No significant change was observed during the course of the study.



**Figure S4. Correlation of tumor mutational burden (TMB) with clinical efficacy. (A)** PFS of subjects by TMB≥6 Muts/Mb versus TMB <6 Muts/Mb. **(C)** OS of subjects by TMB≥6 Muts/Mb versus TMB <6 Muts/Mb. No difference was found between two groups. Percentages of survival patients are shown at indicated time points. Numbers of patients at risk at indicated time points are shown below the x-axis.



**Table S1. Treatment related serious adverse events (SAE).** By the safety data cut-off date of July 31 2018, treatment related SAEs and incidence rate in each cohort are listed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 1 mg/kg (n=15) | 3 mg/kg (n=15) | 10 mg/kg (n=6) | Total (n=36) |
|  | Case | N (%) | Case | N (%) | Case | N (%) | Case | N (%) |
| Total | 3 | 2(13.33) | 1 | 1(6.67) | 3 | 2(33.33) | 7 | 5(13.89) |
| Appetite decreased | 0 | 0(0.00) | 0 | 0(0.00) | 2 | 1(16.67) | 2 | 1(2.78) |
| Lung infection | 0 | 0(0.00) | 1 | 1(6.67) | 0 | 0(0.00) | 1 | 1(2.78) |
| Disease progression | 0 | 0(0.00) | 0 | 0(0.00) | 1 | 1(16.67) | 1 | 1(2.78) |
| Fever | 1 | 1(6.67) | 0 | 0(0.00) | 0 | 0(0.00) | 1 | 1(2.78) |
| Irregular heart beat | 2 | 1(6.67) | 0 | 0(0.00) | 0 | 0(0.00) | 2 | 1(2.78) |

**Table S2. Grade 3 and above treatment related adverse events (TRAE) in each cohort.** By the safety data cut-off date of July 31 2018, treatment related grade 3 and above AE and incidence rate in each cohort are listed. G3, grade 3; G4, grade 4.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Grade ≥3 TRAE | 1 mg/kg | G3 | G4 | 3 mg/kg | G3 | G4 | 10 mg/kg | G3 | G4 | Total | G3 | G4 |
|  | N (%) |  |  | N (%) |  |  | N (%) |  |  | N (%) |  |  |
|  | 5(100.00) | 5 | 0 | 5(100.00) | 5 | 0 | 3(100.00) | 3 | 0 | 13(100.00) | 13 | 0 |
| Hypokalemia | 1(6.67) | 1 | 0 | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 2(5.56) | 2 | 0 |
| ALT increased | 0(0.00) | 0 | 0 | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Amylase increased | 0(0.00) | 0 | 0 | 0(0.00) | 0 | 0 | 1(16.67) | 1 | 0 | 1(2.78) | 1 | 0 |
| DBIL increased | 0(0.00) | 0 | 0 | 2(13.33) | 2 | 0 | 0(0.00) | 0 | 0 | 2(5.56) | 2 | 0 |
| Proteinuria | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Creatine kinase increased | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Serum Creatinine increased | 0(0.00) | 0 | 0 | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Hyperglycemia | 0(0.00) | 0 | 0 | 0(0.00) | 0 | 0 | 1(16.67) | 1 | 0 | 1(2.78) | 1 | 0 |
| Low blood pressure | 0(0.00) | 0 | 0 | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Lipase increased | 1(6.67) | 1 | 0 | 1(6.67) | 1 | 0 | 2(33.33) | 2 | 0 | 4(11.12) | 4 | 0 |
| Kidney disease | 1(6.67) | 0 | 0 | 1(6.67) | 1 | 0 | 0(0.00) | 0 | 0 | 1(2.78) | 1 | 0 |
| Anemia | 2(13.33) | 2 | 0 | 0(0.00) | 0 | 0 | 1(16.67) | 1 | 0 | 3(8.34) | 3 | 0 |

**Table S3. PD-1 receptor occupancy (RO) by JS001 in three dose cohorts.** The mean, median, SD and range of RO of activated T cells (CD3+ CD45RA-), activated CD8 T cells (CD3+ CD8+ CD45RA-) and activated CD4 T cells (CD3+ CD8- CD45RA-) are shown.

|  |  |  |  |
| --- | --- | --- | --- |
| **RO (%)** | **Activated T cell** | **Activated CD8 T cell** | **Activated CD4 T cell** |
| **Cohort** | **Mean** | **SD** | **Median** | **Range** | **Mean** | **SD** | **Median** | **Range** | **Mean** | **SD** | **Median** | **Range** |
| 1 mg/kg (n=13) | 91.66 | 8.59 | 91.91 | 65-100 | 89.68 | 7.35 | 90.16 | 70-100 | 93.04 | 9.65 | 95.44 | 62-100 |
| 3 mg/kg (n=14) | 95.21 | 6.29 | 97.08 | 79-100 | 92.97 | 6.87 | 93.80 | 75-100 | 96.38 | 5.26 | 98.66 | 82-100 |
| 10 mg/kg (n=7) | 97.52 | 1.78 | 96.65 | 95-100 | 95.12 | 4.02 | 95.32 | 88-99 | 98.24 | 1.23 | 97.46 | 97-100 |

**Table S4.** **Subgroup analysis of correlation with clinical efficacy.** Parameters analyzed for correlation with clinical efficacy included PD-L1 expression and TIL in tumor biopsy, ECOG performance score, LDH level, tumor burden at baseline, age, gender, prior lines of treatment. \* PD-L1 positive status was defined as the presence of membrane staining of any intensity in ≥ 1% of tumor cells. \*\* ULN, upper limit of normal for LDH serum level, 250 U/L. \*\*\* Tumor volume was represented by sum of diameters of target lesions.100 mm was used as a cut-off.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristic** | **Value** | **n** | **ORR** | **DCR** |
| **Age** | **≤50** | 16 | 18.8% | 50.0% |
|  | **>50** | 20 | 25.0% | 50.0% |
| **Gender** | **Male** | 20 | 25.0% | 50.0% |
|  | **Female** | 16 | 18.8% | 50.0% |
| **PD-L1 expression** | **+** | 16 | 43.8% | 62.5% |
|  | **-** | 12 | 0.0% | 50.0% |
| **TIL** | **+** | 22 | 31.8% | 59.1% |
|  | **-** | 6 | 0.0% | 50.0% |
| **ECOG** | **0** | 16 | 37.5% | 56.3% |
|  | **1** | 20 | 10.0% | 45.0% |
| **LDH** | **normal** | 20 | 30.0% | 50.0% |
|  | **>ULN** | 16 | 12.5% | 50.0% |
| **Tumor volume** | **<100 mm** | 19 | 36.8% | 68.4% |
|  | **≥100 mm** | 17 | 5.9% | 29.4% |
| **Prior lines of therapy** | **1** | 5 | 20.0% | 60.0% |
|   | **2** | 12 | 25.0% | 58.3% |
|   | **3+** | 19 | 21.1% | 36.8% |
| **Total** |  | 36 | 22.2% | 50.0% |

**Table S5. Tumor mutational burden measurement and correlation with clinical response.** \* Subjects were still alive on July 3, 2018 and OS was not reached for these subjects.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Subject** | **Type** | **TMB value (Muts/Mb)** | **PFS (days)** | **OS (days)** | **Response** |
| 01001\* | Acral Melanoma | 6.40 | 323 | 825 | PR |
| 01002 | Acral Melanoma | 5.40 | 84 | 194 | PD |
| 01003\* | RCC | 4.80 | 364 | 810 | SD |
| 01004 | Acral Melanoma | 7.20 | 401 | 401 | CR |
| 01005 | Melanoma | 6.40 | 82 | 161 | PD |
| 01006 | UC | 9.60 | 50 | 50 | PD |
| 01K1007 | UC | 9.60 | 167 | 401 | SD/uPR |
| 01K1008 | Acral Melanoma | 0.80 | 57 | 425 | PD |
| 01K1011 | Acral Melanoma | 2.40 | 112 | 478 | PD |
| 01K1012 | RCC | 6.20 | 57 | 293 | PD |
| 01K1013 | Melanoma | 4.80 | 133 | 156 | PD |
| 01K1014 | Acral Melanoma | 17.60 | 57 | 292 | PD |
| 1018\* | UC | 152.80 | 658 | 658 | PR |
| 01K3019 | Mucosal Melanoma | 5.40 | 55 | 170 | PD |
| 01020\* | RCC | 6.20 | 147 | 627 | PR |
| 01K3023\* | RCC | 3.20 | 55 | 623 | PD |
| 01K3024 | Melanoma | 1.60 | 225 | 461 | SD |
| 01026 | Acral Melanoma | 2.40 | 83 | 99 | PD |
| 01027 | Melanoma | 1.60 | 83 | 223 | PD |
| 01K3030 | RCC | 7.80 | 225 | 331 | PR |
| 01K3032\* | Mucosal Melanoma | 3.10 | 586 | 586 | PR |
| 01035 | UC | 6.40 | 55 | 163 | PD |
| 01036\* | UC | 3.20 | 222 | 554 | PR |