Supplementary Materials

Activated Lymphocytes and Increased Risk of Dermatologic Adverse Events during Sorafenib Therapy for Hepatocellular Carcinoma

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Table S1. Definition of the combination of markers used for the identification of every lymphocyte population.

| Population | Subpopulation | Combination |
|------------|---------------|-------------|
| T          | T CD4⁺        | CD3⁺CD56    |
| T          | T CD8⁺        | CD3⁺CD56CD4⁻CD8 |
| T          | Treg          | CD3⁺CD56CD4⁻CD8CD25⁻CD127⁻ |
| B          |               | CD3⁻CD56CD19⁺ |
| NK         | CD56⁻bright   | CD56⁻brightCD3⁻ |
| NK         | CD56⁻dim      | CD56⁻dimCD3⁻ |
| NK-like    | CD56⁻bright   | CD56⁻brightCD3⁺ |
| CD3⁺       | CD56⁻dim      | CD56⁻dimCD3⁺ |

T: T cells, T CD4⁺: T helper cells, T CD8⁺: cytotoxic T cells, Treg: Regulatory T cells, NK: Natural Killer cells.

Table S2. List of the fluorophores used in the study.

| Marker | Fluorophore | Reference |
|--------|-------------|-----------|
| CD3    | Pacific Blue| BioLegend, 300431 |
| CD4    | BUV395      | BD Biosciences, 563550 |
|        | AlexaFluor700| BD Biosciences, 557922 |
| CD8    | Brilliant Violet 605| BioLegend, 344742 |
| CD19   | Brilliant Violet 711| BioLegend, 302246 |
|        | PE          | eBioscience, 12-0199-42 |
| CD56   | APC         | eBioscience, 17-0566-42 |
|        | BUV395      | BD Biosciences, 563554 |
| CD25   | PE          | BioLegend, 356134 |
| CD16   | Brilliant Violet 785| BioLegend, 302638 |
| PD-1   | FITC        | BioLegend, 369310 |
| CD69   | PerCP       | BioLegend, 310928 |
| CXCR6  | Brilliant Violet 510| BD Biosciences, 743598 |
| LAG-3  | PE-Cy7      | BioLegend, 369310 |
| CD127  | APC-eFluor780| eBioscience, 47-1278-42 |
| CD39   | Brilliant Violet 510| BioLegend, 328219 |
| Antibody | Clone | Supplier | Catalog Number |
|----------|-------|----------|----------------|
| NKG2D    |       | R&D Systems | FAB139N-100 |
| DNAM-1   | Brilliant Violet 605 | BioLegend | 338323 |
|          | Brilliant Violet 711 | BioLegend | 338334 |
|          | Purified | BD Biosciences | 559786 |
| TIGIT    | Brilliant Violet 605 | BioLegend | 372172 |
| CD96     | BB515 | BD Bioscience | 564774 |
|          | Purified | HyCult Biotech | HM2210-100UG |
| Eomes    | PE-eFluor 710 | eBioscience | 61-4877-42 |
| T-bet    | eFluor660 | eBioscience | 50-5825-80 |
| Viability | VivaFix 353/442 | BioRad | 1351111 |
| IL-10    | eFluor 660 | eBioscience | 50-7108-42 |
| IL-4     | PE     | eBioscience | 12-0086-41 |
| IFN-γ    | Alexa Fluor 488 | eBioscience | 53-7319-41 |
| TNF-α    | Brilliant Violet 785 | BioLegend | 502947 |
| Granzyme B | PE/Cyanine7 | BioLegend | 372213 |
| CD107a   | Brilliant Violet 605 | BioLegend | 328633 |
| CD155    | Purified | ThermoFisher | MA5-13493 |
| IgG F(ab’)2 | Purified | ThermoFisher | 31192 |

In addition, FcR blocking reagent [Milteny Biotech, 130-059-901] was added to all stainings. Sphero™ Rainbow Calibration Particles (8 peaks), 3.0 - 3.4 µm [BD Biosciences, 559123] were acquired after each assay for control calibration.

Staining buffer was composed of dPBS supplemented with 2% FBS and 1mM EDTA.
Figure S1. Histogram representation of CD69, IL-10 and Grnz-B expression of T CD4+ DNAM-1+ and DNAM-1− cells after PMA/Ionomycin stimulation. Histogram representation of CD69, IL-10 and Grnz-B expression of T CD4+ DNAM-1+ and DNAM-1- cells with or without PMA/Ionomycin stimulation. Grnz-B: Granzyme B, PMA: phorbol myristate acetate, ION: Ionomycin.
Figure S2. Histogram representation of T cells cytokines expression. T CD4+ DNAM-1+ and DNAM-1- cells were activated for 72h using different combinations of the following: plate-bound CD3, CD155, CD96, DNAM-1 and soluble CD28, DNAM-1 and IL-15. Then, we evaluated the expression of IFN-γ, TNF-α, IL-4, CD107a, IL-10 and Grnz-B. pCD3: plate-bound anti-CD3, sCD28: soluble anti-CD28, pDNAM-1: plate-bound anti-DNAM-1, pCD155: plate-bound anti-CD155, pCD96: plate-bound anti-CD96.
Figure S3. Cytotoxic ability of lymphocytes co-cultured with autologous pre-activated T CD4+ cells. Cytotoxic capacity of CD4+ effector lymphocytes co-cultured with autologous pre-activated T CD4+ cells, either DNAM-1+ or DNAM-1−, at ratios 1:1 (A), 1:2 (B) and 1:10 (C). pCD3: plate-bound anti-CD3, sCD28: soluble anti-CD28, Sora: Sorafenib, pDNAM-1: plate-bound anti-DNAM-1, pCD155: plate-bound anti-CD155, pCD96: plate-bound anti-CD96.