



**Appendix Table 1. Sample size requirements from power calculations (1-sample, 2-sided equality).**

|                 | <b>2.2.1<br/>IFN-<math>\gamma</math> Stimulation</b> |             | <b>2.2.2<br/>PBMC<br/>Stimulation<br/>(IFN- <math>\gamma</math>)</b> |              | <b>2.2.2<br/>PBMC<br/>Stimulation<br/>(IFN- <math>\gamma</math>+) </b> |              | <b>2.2.3<br/>NK Cell<br/>Stimulation<br/>(IFN- <math>\gamma</math>)</b> |                 | <b>2.2.4<br/>Chemotherapy Stimulation</b> |   |  |  | <b>2.2.5<br/>Irradiation Stimulation</b> |        |        |
|-----------------|--|-------------|--|--------------|--|--------------|---|-----------------|---|---|--|--|--|--------|--------|
|                 | All time points                                      | 3 Days Only | All time points  | 5 & 10 Hours | All time points  | 5 & 10 Hours | IFN- $\gamma$   | IFN- $\gamma$ + | All t'x (6 & 8 days)                      | T <sup>+</sup> /P <sup>+</sup> (6 & 8 days) | T <sup>+</sup> & T <sup>+</sup> (6 & 8 days) | P <sup>+</sup> & P <sup>+</sup> (6 & 8 days) | 1 day                                    | 3 days | 5 days |
| <b>HLA-ABC</b>  | /  | 5           | /  | 3            | 3  | 3            | 8-15  | 3               | /   | 8-13  | 3-18   | 3-20   |  | 3-10   |        |
| <b>HLA-Bw4</b>  | 14-15  | 15          | 3  | 3            | 3  | 3            | 3-18  | 6-7             | 3-13                                      | 10-12                                       | 4-13   | 3-8  | 3-6                                      |        |        |
| <b>HLA-C</b>    | 3  | 3           | 3-13   | 3            | 3  | 3            | /   | 3               | /   | 4-7   | 3-5  | 3-10   |  |        | 3-6    |
| <b>HLA-E</b>    | 3-4  | 4           | 3-6  | 3            | 3  | 3            | /   | 3               | /   | 5-9   | 4-9  | /  |  |        |        |
| <b>HLA-G</b>    | /  | 7           | 3  | 3            | 3-4  | 3            | 3   | 3               | 3-9                                       | 6-8   | 3-6  | 3-9  | 3  |        |        |
| <b>PD-L1</b>    | 3  | 3           | 3-8  | 3-4          | 3  | 3            | 5-7   | 5               | 3-12                                      | 4-10  | 5-10   | 3-4  | 4-7                                      | 3-11   | 4-6    |
| <b>MIC-A/B</b>  |  |             | /  | 3            | 3  | 3            | 3   | 3               | /   | /   | /  | /  | 9-14                                     |        |        |
| <b>Fas</b>      | 3  | 3           | 3-6  | 3-6          | 8-13   | 11-13        | /   | 3               | /   | 5-13  | /  | /  | 3-5                                      | 3-6    | 4-6    |
| <b>HLA-F</b>    | /  | 4           | 3-18   | 3-9          | 6-10   | 6-7          | /   | 9-13            | /   | 5-12  | 5-12   | 3-6  | 3-8                                      | 3-9    | 5-6    |
| <b>TRAIL-R1</b> |  |             | 3-5  | 3-4          |  |              | /   | 8-9             | /   | /   | /  | /  | 3-5                                      |        |        |
| <b>TRAIL-R2</b> |  |             | /  | 3 (5 hrs)    | 3-20   | 13-20        |   |                 | /   | 4-12  | 3-10   | /  | 4-16                                     |        | 10-15  |

 = minimum sample size reached for 80% power (5% type I error rate)


 = no trend in data

/ = >20 samples required

3-x = sample size reached for some but not all within that condition

Appendix Table 2. Sample size requirements from power calculations (2-sample, 2-sided equality).

|   | 2.2.2<br>PBMC<br>Stimulation |             | 2.3.1<br>HLA Blockade<br>(IFN- $\gamma^+$ ) |       | 2.3.2<br>PD-1/PD-L1 Blockade<br>(IFN- $\gamma^+$ ) |                |       | 2.3.5<br>Chemotherapy |       |      |      |       |
|---|------------------------------|-------------|---|-------|--|----------------|-------|-----------------------|-------|------|------|-------|
|   | All time<br>points           | 10<br>hours | DT9   | W6/32 | Anti-<br>PD-1                                      | Anti-<br>PD-L1 | Combo | Unstim.               | A549  | P    | T    | T+P   |
| <b>NK cells</b>   | 3-12                         | 12          |   |       |  |                |       |                       |       |      |      |       |
| <b>NKG2A<sup>+</sup></b>  |                              |             | /   | 7     |  |                |       |                       |       |      |      |       |
| <b>KIR<sup>+</sup></b>  |                              |             | /   | /     |  |                |       |                       |       |      |      |       |
| <b>KIR2DL2/L3<sup>+</sup></b>   |                              |             | /   | /     |  |                |       |                       |       |      |      |       |
| <b>KIR3DL1<sup>+</sup></b>  |                              |             | /   | /     |  |                |       |                       |       |      |      |       |
| <b>KIR<sup>-</sup> NKG2A<sup>-</sup> PD-1<sup>+</sup></b>                   |                              |             |   |       | /  | 13             | 5     |                       |       |      |      |       |
| <b>KIR<sup>-</sup> NKG2A<sup>-</sup> PD-1<sup>+</sup> NKG2D<sup>+</sup></b> |                              |             |   |       | /  | /              | 9     |                       |       |      |      |       |
| <b>Act<sup>+</sup> KIR<sup>+</sup></b>                                      |                              |             |   |       |  |                |       | 11-5                  | 14-20 | /    | 4-13 | 11-17 |
| <b>Act<sup>+</sup> KIR<sup>-</sup></b>                                      |                              |             |   |       |  |                |       | 3                     | 6-8   | 7-17 | 3-5  | 3-7   |

 = no statistical tests

 = minimum sample size reached for 80% power (5% type I error rate)

/ = >20 samples required

3-x = sample size reached for some but not all within that condition

**Appendix Table 3. Experimental conditions of assumed normality with one time-point or condition that did not pass  $\alpha=0.05$  (Shapiro-Wilk normality test).**

| Section | Title   | Ligand   | Time-Point/Condition    |
|---------|---|--|-------------------------|
| 3.1.1   | <i>Exposure to IFN-<math>\gamma</math> induces rapid evolution of death receptors and activating and inhibitory ligands on A549 cells</i>                                   | HLA-E  | Day 2 (p=0.0246)        |
|         |   | HLA-G  | Day 2 (p=0.0048)        |
|         |   | HLA-ABC  | Day 1 (p=0.0064)        |
| 3.1.2   | <i>PBMC pressure induces rapid evolution of death receptors and activating and inhibitory ligands on A549 cells</i>   | HLA-G (IFN- $\gamma$ <sup>-</sup> )  | 3 hours (p=0.0330)      |
|         |   | HLA-C (IFN- $\gamma$ <sup>-</sup> )  | 10 hours (p=0.0368)     |
|         |   | HLA-ABC (IFN- $\gamma$ <sup>+</sup> )  | 10 hours (p=0.0439)     |
|         |   | HLA-E (IFN- $\gamma$ <sup>+</sup> )  | 10 hours (p=0.0086)     |
|         |   | MIC-A/B (IFN- $\gamma$ <sup>+</sup> )  | 3 hours (p=0.0138)      |
|         |   | TRAIL-R2 (IFN- $\gamma$ <sup>+</sup> )   | 10 hours (p=0.0271)     |
|         |   | NK cells   | Unstimulated (p=0.0030) |
| 3.1.4   | <i>Palbociclib and trametinib chemotherapy induces death receptors and activating and inhibitory ligand upregulation in a dose- and time-dependent manner on A549 cells</i> | HLA-C  | 8 days (p=0.0442)       |
|         |   | HLA-E  | 2 days (p=0.0251)       |
| 3.2.1   | <i>HLA ligands' blockade on A549 cells induces greater response of cognate receptor-positive NK cells</i>   | KIR3DL1 <sup>+</sup> NK cells  | W6/32 (p=0.0490)        |
|         |   | KIR2DL2/L3 <sup>+</sup> NK cells   | A549 (p=0.0332)         |
| 3.2.2   | <i>Blocking the PD-1/PD-L1 axis induces greater response of PD-1<sup>+</sup> NK cells</i>   | PD-1 <sup>+</sup> NKG2D <sup>+</sup> KIR <sup>-</sup><br>NKG2A <sup>-</sup> NK cells | Unstimulated (p=0.0364) |
| 3.3.1   | <i>The subset of NK cells responding to a tumor can be predicted by the tumor cell phenotype post-chemotherapy treatment</i>  | TRAIL <sup>+</sup> KIR2DL2/L3 <sup>+</sup><br>KIR3DL1 <sup>+</sup>                   | Palbociclib (p=0.0360)  |