Supplementary table 1. Criticality matrices for PHA.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Occurrence** | | | |  |  |
|  |  | **1** | **2** | **3** | **4** |  |  |
| **Severity** | **1** | 1 | 2 | 3 | 4 | **1** | **Detection** |
| **2** | 4 | 8 | 12 | 16 | **2** |
| **3** | 9 | 18 | 27 | 36 | **3** |
| **4** | 16 | 32 | 48 | 64 | **4** |

|  |  |  |
| --- | --- | --- |
| **CRITICALITY SCALE** | | |
| **Index** | **Level of criticality** | **Decision** |
| 1-17 | Acceptable | No action required |
| 18-35 | Tolerable | Appropriate management should be implemented |
| 36-64 | Unacceptable | Mitigation measures must be implemented |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SCALE USED FOR ESTIMATION OF SCORE ASSOCIATED WITH EACH IDENTIFIED RISK** | | | | | |
| **SEVERITY** | | | **LIKELIHOOD** | | |
| **score** | **Impact on GMP product quality**  **(S)** | | **Score** | **Probability of occurrence**  **(O)** | **Probability of Detection**  **(D)** |
| 1 | Minor | No significant impact on CQA | 1 | Remote event | Event frequently detectable / before CQ test conclusion |
| 2 | Major | Minimal and reversible impact on CQA | 2 | Occasional event | event easily detectable with appropriate control system |
| 3 | Critical | Critically alter CQA (potential regulatory incompliance) | 3 | Frequent event | Event for which control systems can identify failures/ after CQ conclusion |
| 4 | Catastrophic | Highly alter the product safety / need for recall(regulatory incompliance) | 4 | Very frequent event | Undetectable event |

CQA, critical quality attributes.

Supplementary table 2. Multicolor flow cytometry for investigational medicinal product characterization: type and quantity of antibodies per assessment.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Antibody staining panel to assess:** | | | | **Treg cells enumeration [Trucount]** | **FoxP3**  **expression** | **Contami-nating cells** |
|  | CD4 | FITC | BD Multi-Clone | SK3, SK4 | 20 µL | 20 µL | 20 µL |
|  | CD8 | PE | BD Pharmingen | HIT8a | 20 µL | / | / |
|  | IgG2a kappa  *or*  FoxP3 | PE  PE | eBioscience  eBioscience | eBR2a  PCH101 | / | 0.5 µL  5 µL | / |
|  | CD56 | PE | BD Pharmingen | B159 | / | / | 5 µL |
|  | 7-AAD | / | BD | / | 20 µL | 20 µL | 20 µL |
|  | CD127 | PE-Cy7 | BD Pharmingen | HIL-7R-M21 | 5 µL | 5 µL | / |
|  | CD19 | PE-Cy7 | BD Pharmingen | HIB19 | / | / | 5 µL |
|  | CD25 | APC | BD Pharmingen | M-A251 | 20 µL | 20 µL | / |
|  | CD8 | APC | BD Pharmingen | M-A251 | / | / | 20 µL |
|  | CD45 | APC-H7 | BD Pharmingen | 2D1 | 5 µL | 5 µL | 5 µL |



Supplementary figure 1. Negative control for FoxP3 staining.

For assessment of FoxP3 expression on Treg cells, postenrichment and postexpansion samples were stained with Foxp3 PE or the corresponding isotype control, and analyzed according to the gating strategy described in Figure 2. A, B: Plots displaying IgG2a kappa isotype control staining of postenrichment (A) and postexpansion (B) samples. C, D: Overlay of the isotype-stained control (blue) onto the corresponding FoxP3-stained sample (red, inset) for postenrichment (C) and postexpansion (D) samples. Representative images of the samples from patient KD2 depicted in Figure 2 are shown.