**Supplementary Information**

Liposomal formulation of an organogold complex enhancing its activity as antimelanoma agent – in vitro and in vivo studies

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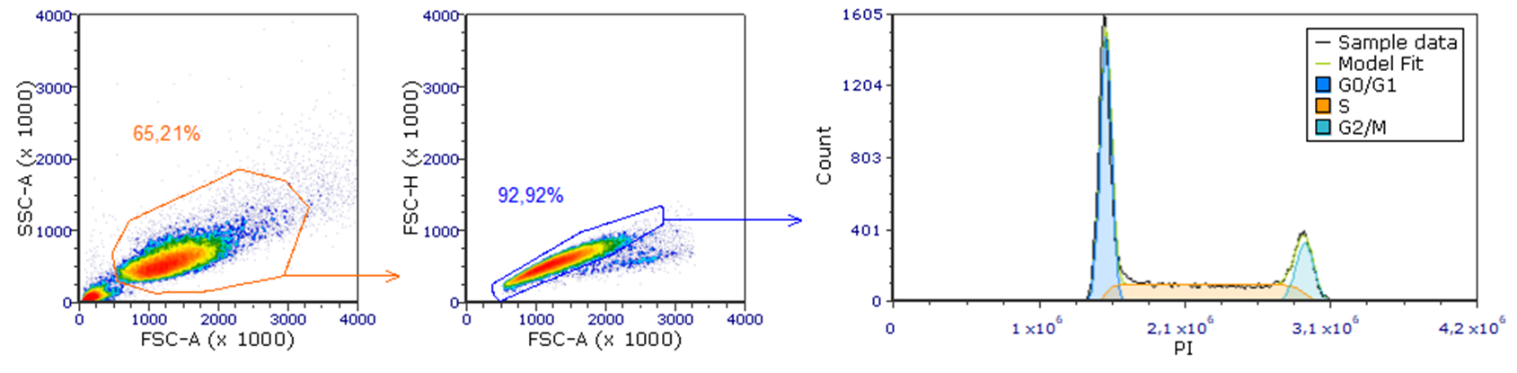
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**Supplementary Results**

1. **Cell cycle analysis**



**Figure S1.** Gating strategy for cell cycle analysis by flow cytometry. Debris and doublets were excluded before assessing propidium iodide (PI) fluorescence. A cell cycle histogram was automatically generated for each sample using the MultiCycle AV in FCS Express 7 Software (DeNovo Software).

A collage of graphs

Description automatically generated

**Figure S2.** Representative plots of gated B16F10, A375 and MNT-1 melanoma cells in the G0/G1, S, and G2/M phases of cell cycle in the absence (Control) or presence of DTIC at 70 μM (DTIC) or ST004 in free (Free-ST004) or liposomal (LIP-ST004) forms. A cell cycle histogram was automatically generated for each sample using the MultiCycle AV in FCS Express 7 Software (DeNovo Software).

A screenshot of a video game

Description automatically generated

**Figure S3.** Therapeutic effect of tested ST004 formulations in a subcutaneous murine melanoma model. Tumor induction was performed by a s.c. injection of 1.3×105 B16F10 cells/mouse. Mice received i.v. injections of the formulations at a dose of 3.5 mg/kg of body weight, five consecutive times, once per day. Three experimental groups were established: Control (induced and non-treated mice); Free-ST004; LIP-ST004 (liposomal formulation of ST004, DMPC:DOPE:DSPE-PEG). **(a)** Experimental design, **(b)** Average animal weight, and **(c)** Tumor volume evolution. Tumor volumes were calculated according to the formula: V (mm3) = (L × W2)/2, where L and W represent the longest and shortest axis of the tumor, respectively. Results are expressed as mean ± SEM (n = 4-5).

**Table S1.** Tissue index and hepatic biomarkers of the subcutaneous murine melanoma model.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Tissue Index (AVG ± SEM)** | | | | |  | **Hepatic Biomarkers (AVG ± SEM)** | |
|  | **Liver** | **Spleen** | **Lungs** | **Kidneys** |  | **AST (U/L)** | **ALT (U/L)** |
| **Naïve** | 22.1 ± 0.6 | 5.1 ± 0.1 | 6.3 ± 0.6 | 10.7 ± 0.5 |  | 19.2 ± 3.5 | 6.1 ± 2.6 |
| **Control** | 21.3 ± 1.3 | 5.1 ± 0.2 | 6.5 ± 0.3 | 10.7 ± 0.2 |  | 9.6 ± 4.0 | 5.7 ± 2.3 |
| **DTIC** | 24.4 ± 0.4 | 5.4 ± 0.1 | 7.2 ± 0.2 | 11.2 ± 0.1 |  | 26.8 ± 4.6 | 4.1 ± 2.2 |
| **Free-ST004** | 23.4 ± 0.3 | 5.3 ± 0.2 | 7.4 ± 0.1 | 10.4 ± 0.1 |  | 24.8 ± 5.3 | 6.6 ± 3.6 |
| **LIP-ST004** | 23.6 ± 0.5 | 6.4 ± 0.3 | 7.8 ± 0.3 | 10.7 ± 0.1 |  | 21.5 ± 10.8 | 7.4 ± 1.9 |

**Table S2.** Tissue index and hepatic biomarkers of the metastatic murine melanoma model.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tissue Index (AVG ± SEM)** | | | | | | **Hepatic Biomarkers (AVG ± SEM)** | | |
|  | **Liver** | **Spleen** | **Lungs** | **Kidneys** | **Brain** |  | **AST (U/L)** | **ALT (U/L)** |
| **Control** | 20.6 ± 0.3 | 5.5 ± 0.2 | 7.7 ± 0.2 | 10.9 ± 0.1 | 12.6 ± 0.4 |  | 12.2 ± 1.6 | 9.6 ± 2.5 |
| **DTIC** | 20.9 ± 0.4 | 5.5 ± 0.1 | 8.1 ± 0.1 | 11.0 ± 0.1 | 12.1 ± 0.4 |  | 4.8 ± 0.7 | 6.1 ± 0.4 |
| **Free-ST004** | 18.2 ± 0.5 | 6.0 ± 0.4 | 8.0 ± 0.4 | 11.6 ± 0.4 | 11.7 ± 0.7 |  | 12.2 ± 5.0 | 3.5 ± 0.7 |
| **LIP-ST004** | 20.8 ± 0.5 | 6.4 ± 0.2 | 7.4 ± 0.3 | 10.6 ± 0.3 | 11.9 ± 0.6 |  | 21.4 ± 8.8 | 5.2 ± 1.6 |