

Sensitivities of anti-cancer drugs to cancer spheroid growth in the cell array three-dimensional culture system Cell-able™ Oncology

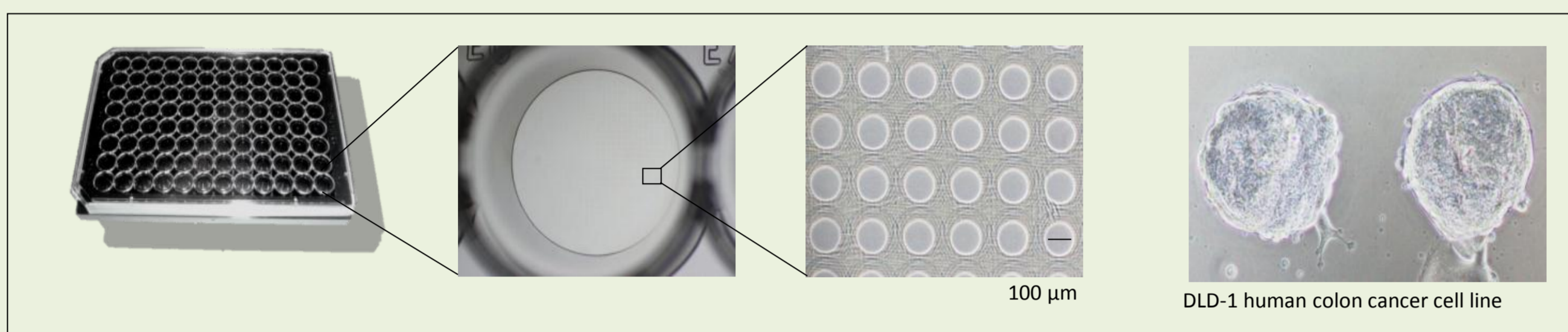
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Abstracts

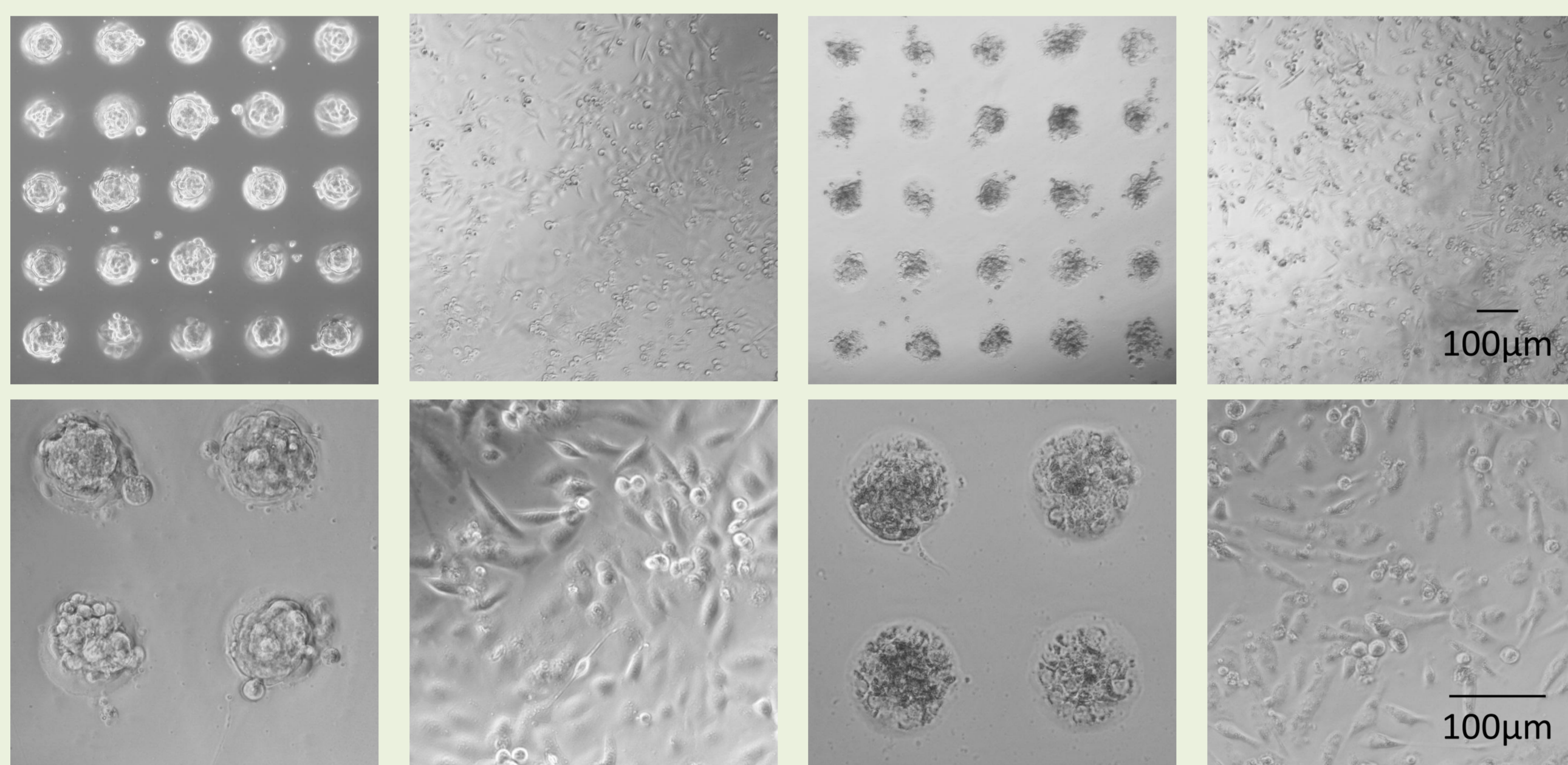
Recently, the cell biology field has come to appreciate the dissimilarity between two- (2D) and three-dimensional (3D) environments in which cells routinely operate in vivo. Efficacy of most anti-cancer drugs have been evaluated in 2D environment but it must be desirable to evaluate it in 3D environment. We developed a novel 3D cell culture system named Cell-able™ Oncology utilizing photo-sensitive materials that change to hydro-gel after UV irradiation and the optimized molecular design not to allow the cells adhere to the hydro-gel. In this study, we demonstrated that the cancer spheroids formed on the Cell-able™ Oncology in serum-free and serum-containing media showed chemo-resistant, high expression of stem cell markers and drug transporters compared to that on the 2D monolayer plate. Thus, the spheroids grown on the Cell-able™ Oncology could provide novel insight to the pharmacodynamics and pharmacokinetics in the anti-cancer drug research and development.

Cell culture in 2D and 3D environments

Human prostate cancer cell line, DU145 were cultured on the Cell-able™ or collagen-coated plates as the 2D environment in RPMI-1640 containing 10% FCS (serum media) or serum-free ReproFF2 (ReproCELL) (stem media). The Cell-able™ Oncology has arrayed cell attachment areas of circle 100 μm in diameter (see below) on each well of the culture plate.

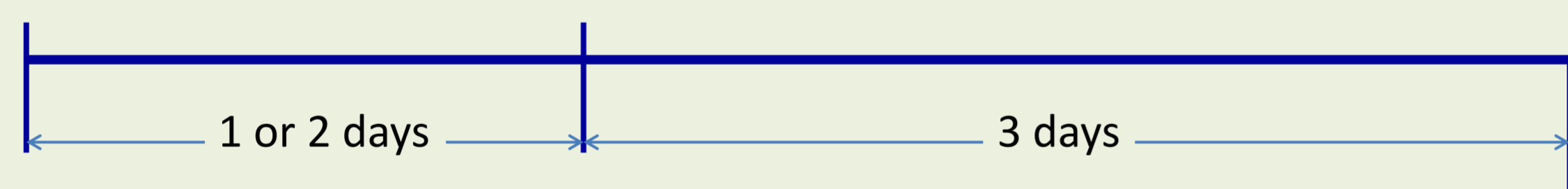


The cancer cells were seeded on the Cell-able™ and cultured for 5 days. The spheroid formed on the circular cell-attachment areas and the morphology was quite different from that of 2D plates as shown below;



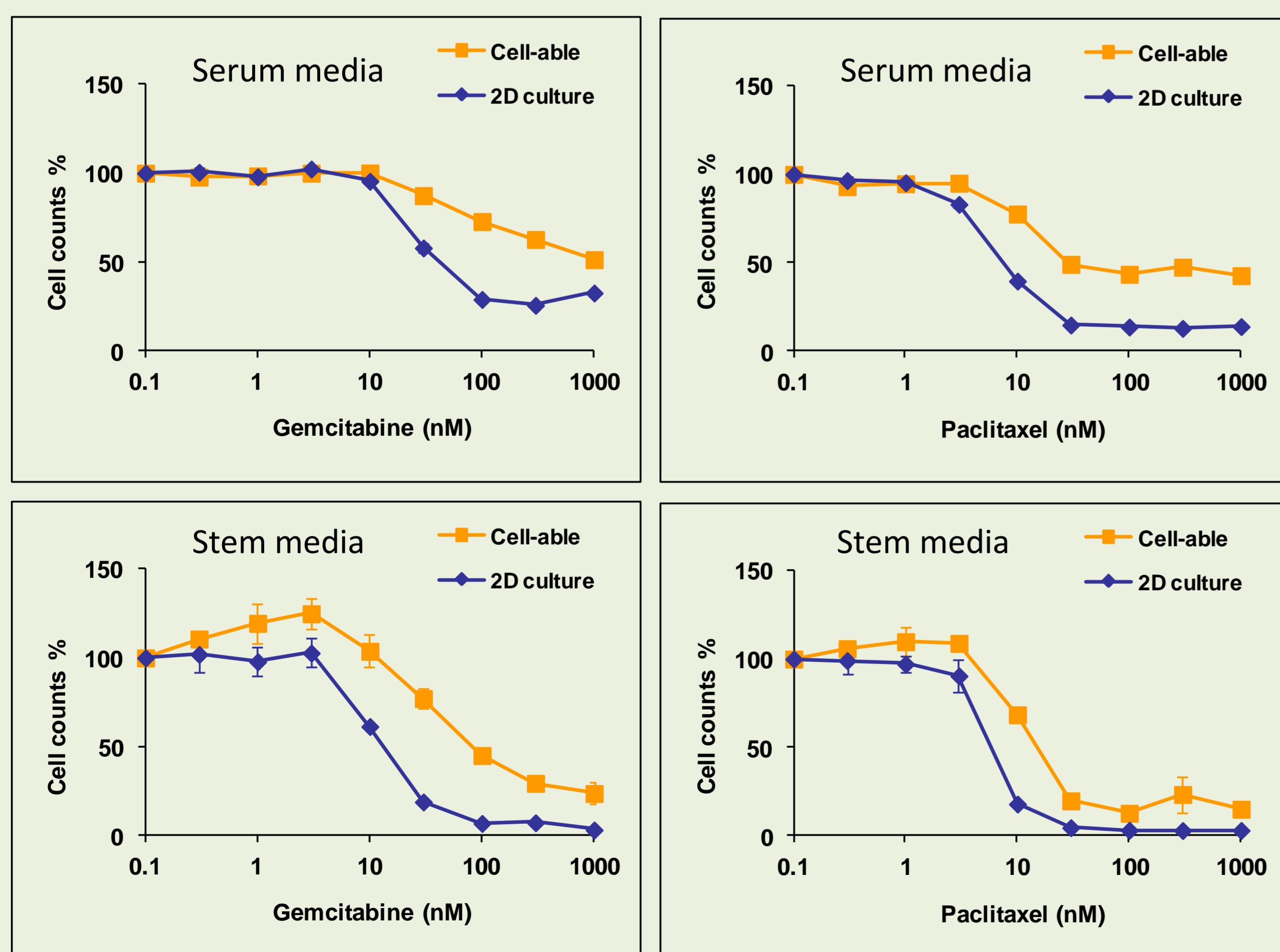
Experimental protocols

Seeding DU145 Drug or vehicle



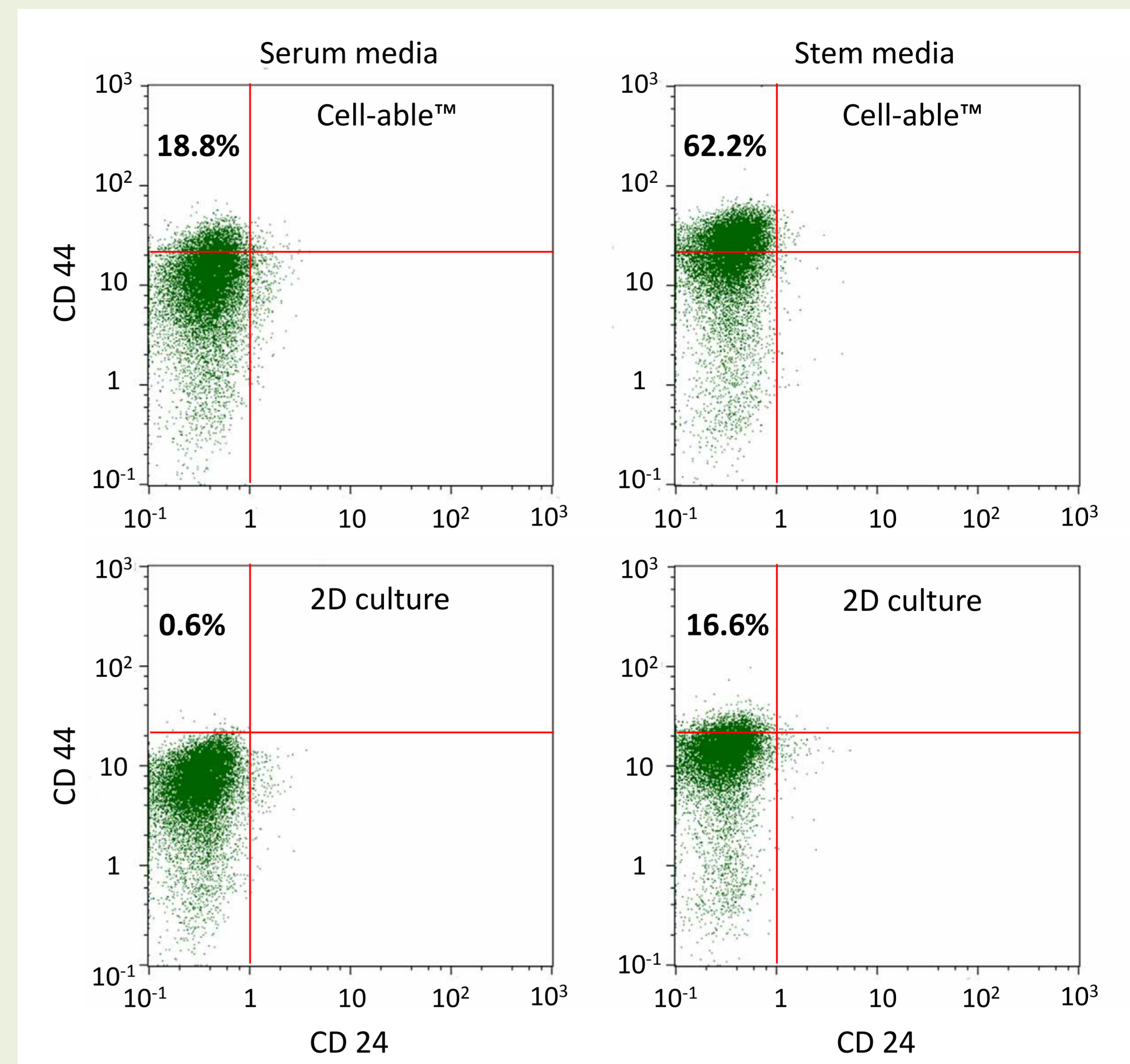
- Groups;
1. Cell-able™ culture in RPMI-1640 supplemented with 10% FCS (serum media)
 2. 2D culture in RPMI-1640 supplemented with 10% FCS (serum media)
 3. Cell-able™ culture in ReproFF2 (stem media)
 4. 2D culture in ReproFF2 (stem media)
- Drug sensitivity analysis: FACS analysis, mRNA expression, CDFDA exclusion

Drug sensitivities of cancer spheroids



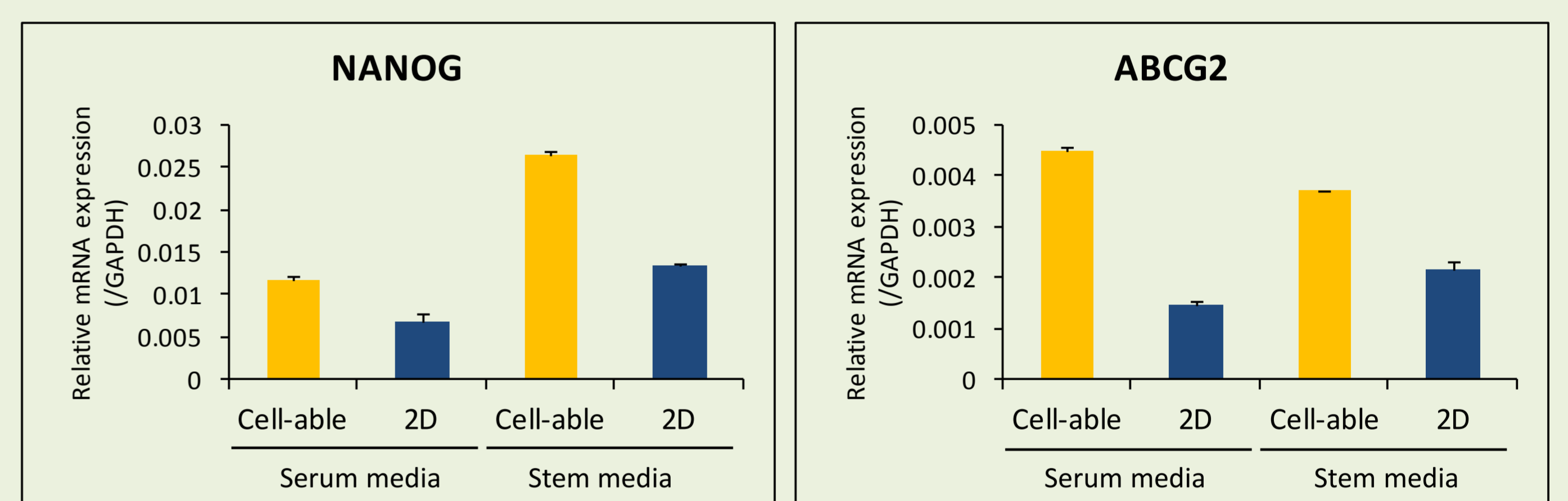
Chemo-resistance of the spheroids grown on the Cell-able™ against gemcitabine (left) and paclitaxel (right) in the serum and stem media compared to 2D culture. Each drug was treated for 3 days and the cell counts were determined by ATP luciferase method (for serum media) or DAPI staining quantified by high content screening assay (for stem media)

Increased CD44^{high}/CD24^{low} cell population in spheroids



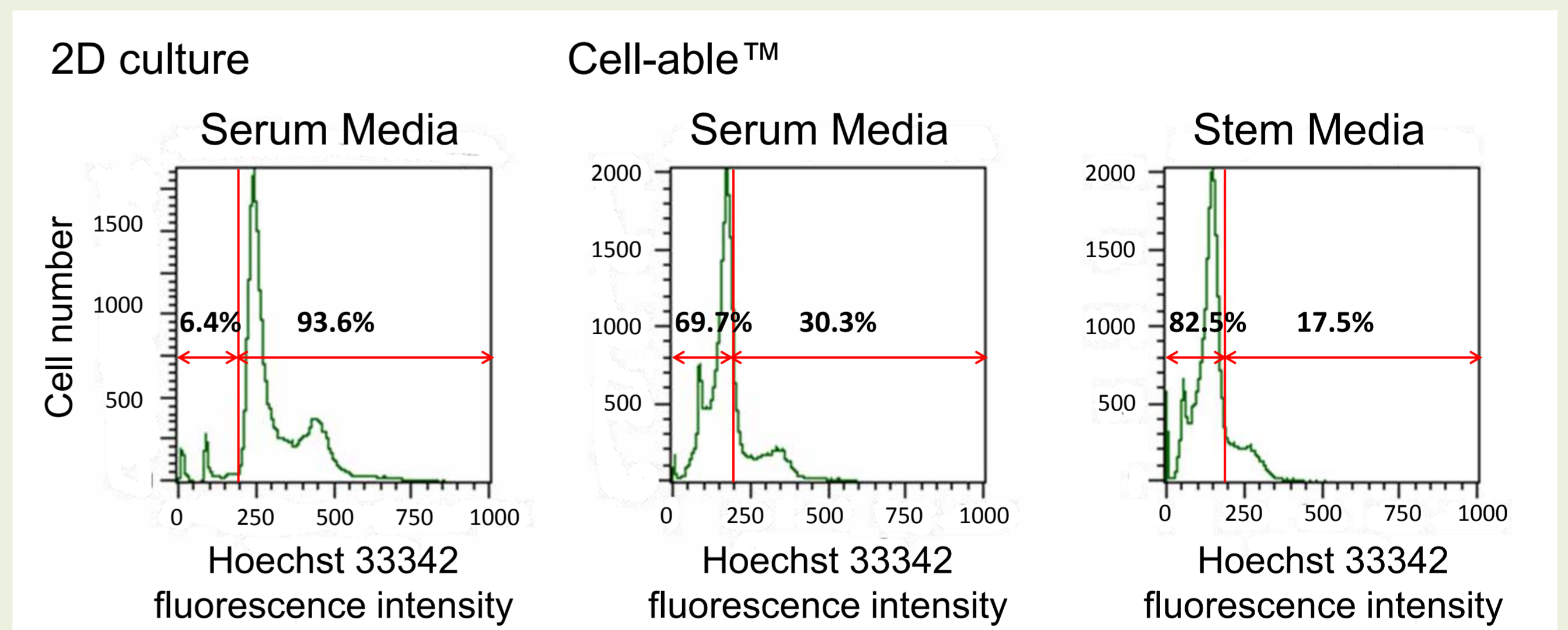
The presence of CD44 and CD24 surface markers of DU145 spheroid grown on Cell-able™ were compared to 2D culture by Fluorescence-activated cell sorting (FACS) analysis. Cells were cultured in serum and stem media and labeled with anti-human CD24-FITC antibody (Invitrogen) and anti-human CD44-APC antibody (BD Biosciences). Samples were analyzed with MACSQuant® Analyzer (Miltenye Biotec).

High expression of mRNA for stem cell markers in spheroids



High expression of mRNA for stem cell markers, Nanog and Abcg2 in spheroids grown on Cell-able™ compared with the 2D culture. Total RNA was extracted from the cultivated cells using (Takara Bio). Complementary DNA was synthesized from total RNA using Mix. Quantitative real time polymerase chain reaction (qRT-PCR) analysis was performed using a Real time PCR with TaqMan Gene Expression Assay reagent and probes (Applied Biosystems). The probe: Nanog (Hs04260_366_g1); Abcg2 (Hs0105370_m1).

Spheroids are stained weakly by Hoechst33342



The fluorescence intensity of DU145 spheroid grown on Cell-able™ stained by Hoechst33342 was compared to 2D culture by Fluorescence-activated cell sorting (FACS) analysis. Cells were cultured in serum and stem media and stained with Hochst33342 (sigma). Samples were analyzed with MACSQuant® Analyzer (Miltenye Biotec).

Conclusions

- The spheroid formation on Cell-able™ lead to the chemo-resistance against gemcitabine and paclitaxel.
- The chemo-resistance was associated with high population of stem cells (CD44^{high}/CD24^{low}) and high expression of stem cell markers, NANOG and ABC transporters ABCG2 and ABCB1.
- The combination of Cell-able™ and the stem media conferred high stem cell population, which may be suitable for drug research and development of drugs targeting the cancer stem cells.
- The pharmacodynamic and pharmacokinetic studies using the cancer stem cell-rich spheroids could provide meaningful information relevant to the clinical conditions.