

Supplementary Table 3: Serial transplantation assay results of SCC-25 derived EpCAM+/ABCG2+ cells

Cell source	Cell dose	Tumor/injection	CSC frequency	Cell source	Cell dose	Tumor/injection	CSC frequency
EpCAM+/ABCG2- (cisplatin+ group)	1 × 10 ²	0/10	1/5.1 × 10 ⁵	EpCAM+ ABCG2+ (Secondary Transplant, Cisplatin+)	1 × 10 ²	0/5	1/1278
	1 × 10 ³	0/10			5 × 10 ²	2/10	
	1 × 10 ⁴	1/15			1 × 10 ³	7/10	
	1 × 10 ⁵	2/15			1 × 10 ⁴	5/5	
EpCAM+ABCG2+ (cisplatin+ group)	1 × 10 ²	5/10	1/125	EpCAM+ ABCG2+ (Tertiary transplant, Cisplatin+)	1 × 10 ²	0/5	1/1086
	5 × 10 ²	10/10			5 × 10 ²	2/10	
	1 × 10 ³	5/5			1 × 10 ³	8/10	
	1 × 10 ⁴	5/5			1 × 10 ⁴	10/10	
EpCAM+/ABCG2- cells (control)	1 × 10 ²	0/10	1 × 1.1 × 10 ⁶	EpCAM+ABCG2+ (Cisplatin++)	1 × 10 ²	7/10	1/140
	1 × 10 ³	0/10			5 × 10 ²	9/10	
	1 × 10 ⁴	0/10			1 × 10 ³	10/10	
	1 × 10 ⁵	1/15			1 × 10 ⁴	5/5	
EpCAM+ABCG2+ (control)	1 × 10 ²	0/10	1/1149	EpCAM+/ABCG2- (Cisplatin++)	1 × 10 ²	0/10	1/3.7 × 10 ⁵
	5 × 10 ²	4/10			5 × 10 ²	0/10	
	1 × 10 ³	6/10			1 × 10 ³	1/15	
	1 × 10 ⁴	5/5			1 × 10 ⁴	3/15	
EpCAM+ABCG2+ (Secondary transplant, cisplatin+ + group)	1 × 10 ²	0/5	1/1081	CD44+ALDH1+cells (Cisplatin++)	1 × 10 ²	1/5	1/769
	5 × 10 ²	0/5			5 × 10 ²	4/10	
	1 × 10 ³	1/5			1 × 10 ³	4/5	
	1 × 10 ⁴	2/5					
EpCAM+ABCG2+ (Secondary transplant, cisplatin+ + group)	1 × 10 ²	0/5	1/7.7 × 10 ⁵	CD44+ALDH1+cells (Cisplatin+)	1 × 10 ²	1/5	1/909
	5 × 10 ²	0/10			5 × 10 ²	3/10	
	1 × 10 ³	0/10			1 × 10 ³	4/5	
	1 × 10 ⁴	2/15					
EpCAM+/ABCG2- (Secondary transplant, cisplatin+ + group)	1 × 10 ³	0/8	1/7.7 × 10 ⁵	CD44+/ALDH1+ cells (Control)	1 × 10 ²	0/5	1/1519
	1 × 10 ⁴	0/10			5 × 10 ²	1/5	
	1 × 10 ⁵	2/15			1 × 10 ³	3/5	

To perform serial transplantation assay, the NOD/SCID mice bearing primary subcutaneous tumors were sacrificed to obtain the tumors, which were then dissociated to obtain single tumor cell suspension. These tumor cells were subjected to immunomagnetic or flow cytometry sorting (CD44+/ALDH1+ cells) and the sorted cells were injected subcutaneously to mice to obtain secondary tumors. Then the protocols for the in vivo limiting dilution assay were followed to obtain the CSC frequency of the secondary transplants. The secondary transplant tumors were then subjected to another round of in vivo limiting dilution assay to obtain the tertiary tumor transplant, and their CSC frequency.