The Figures show:

Figure 1

Schematic representation of the DLL3 ECD / truncated DLL3 constructs expressed on CHO cells for epitope mapping. The transmembrane and the intracellular domain are derived from EpCAM. See Example 1.

Figure 2

Epitope mapping of the anti-DLL3 antibody constructs. Examples of bispecific antibody constructs recognizing the EGF-3 domain and the EGF-4 domain, respectively, as detected by epitope mapping. The x-axis depicts PE-A (PE = phycoerythrin, A = signal area), and the y-axis depicts counts. See Example 2.

Figure 3

Cross-Reactivity of anti-DLL3 antibody constructs as detected by flow cytometry: binding to human and macaque DLL3 and CD3. The x-axis depicts FL2-H, and the y-axis depicts counts. Flow cytometry histograms are shown for anti-DLL3 antibody binding to CHO cells transfected with human DLL3 and CD3-expressing human T cell leukemia cell line HPB-all (Fig. 3A), CHO cells transfected with macaque DLL3 and cynomolgus CD3-expressing T cell line LnPx 4119 (Fig. 3B), and human DLL3 positive human lung carcinoma cell line SHP-77) and CHO cells transfected with artificial human DLL3 isoform (Fig. 3C). See Example 5.

Figure 4

Analysis of anti-DLL3 antibody constructs by flow cytometry: non-binding to human paralogues DLL1 and DLL4. The x-axis depicts FL1-H, and the y-axis depicts counts. See Example 6.

Figure 5

Stability of anti-DLL3 antibody constructs after incubation for 96 hours in human plasma. See Example 11.

Figure 6

Potency gap between the monomeric and the dimeric isoform of the anti-DLL3 antibody constructs. See Example 15.

Figure 7

In vitro internalization assay, carried out as described in Example 16. Antibody construct was pre-incubated on target cells (SHP-77) in the absence of T cells in order to measure loss of antibody construct due to internalization. Results suggest that no significant internalization occurs. The results achieved with a control target which is well known for its internalization are shown for comparison at the right.

Figure 8

Results of the mouse xenograft efficacy study of the SHP-77 model (Fig. 8A) and the WM266-4 model (Figure 8B). The tumor volume is plotted against the time. See Example 18.

Figure 9

Pharmacokinetics of different HLE antibody constructs (albumin fusion in Fig. 9A and Fc fusions in Fig. 9B) as determined in Example 20.