**Background**

CD24 is a small, heavily-glycosylated glycosylphosphatidylinositol (GPI)-anchored protein. It is overexpressed in a variety of adult solid tumors, including breast, ovarian, pancreatic, and colorectal cancers, as well as pediatric cancers such as neuroblastoma, Wilms tumor, and desmoplastic small round cell tumor (DSRCT). In normal tissue, CD24 is expressed transiently during development but is largely absent from mature, differentiated cells. Functionally, CD24 serves as a “don’t eat me” signal to macrophages, binding to Siglec-10 and preventing phagocytosis. It is known as a marker of cancer cell stemness and dysregulates numerous signaling pathways involved in proliferation, invasion, and metastasis. The presence of CD24 has been strongly correlated to poor clinical outcome for multiple tumor types. For these reasons, CD24 is a strong candidate for targeting with T cell-engaging bispecific antibodies (BsAbs).

**Methods**

We used immunohistochemical (IHC) staining on patient specimens and flow cytometry on cancer cell lines to evaluate CD24 expression in a variety of tumor types. We then generated a panel of CD24xCD3 BsAbs of different formats including a humanized IgG-[L]-scFv BsAb and assessed purity and stability using high-performance liquid chromatography. To evaluate the ability of the BsAbs to engage T cells against CD24-expressing tumor cell lines, we performed standard chromium release assays. We used immunocompromised mice bearing luciferase-transduced intraperitoneal xenografts to test the ability of the BsAbs to redirect T cells to CD24-expressing tumor cells in vivo, and monitored tumor growth using in vivo bioluminescent imaging.

**Results**

CD24 was found to be strongly expressed by DSRCT, NB, rhabdomyosarcoma, Ewing sarcoma, mesothelioma, liver cancer, breast cancer, ovarian cancer, and pancreatic cancer. CD24xCD3 BsAbs had high purity (<90%) and were stable for several weeks at 40°C. The humanized CD24xCD3 IgG-[L]-scFv BsAb retained binding ability, indicating that the humanization process did not affect its affinity for CD24 on tumor cells. All the CD24xCD3 BsAbs induced T cell-mediated cytoxicity against DSRCT cell lines in vitro. However, as we have shown previously with BsAbs targeting other tumor antigens, the IgG-[L]-scFv format is superior in vivo, completely ablating established intraperitoneal DSRCT and ovarian cancer xenografts.

**Conclusions**

CD24 is expressed on a variety of solid tumors and is a viable target for T cell-engaging BsAbs. A novel humanized CD24xCD3 BsAb built on an IgG-[L]-scFv platform is effective at clearing disseminated intraperitoneal CD24-positive xenograft tumors. This strategy warrants further study and could eventually be tested in human trials for patients with advanced tumors.

**REFERENCES**
