western blot experiments, as well as studies with siRNA and transfected cells. JAM-A is a type I transmembrane protein that belongs to the immunoglobulin superfamily. JAM-A, one of the protein component of the tight junctions, was not known to play a role in tumor cell proliferation prior to this study. Our results demonstrate that functional and proteomic approaches can be successfully combined to identify new antibody target molecules on tumor cells. This innovative strategy appears promising to develop new antibody-based therapies against cancers.

**523** POSTER

**Combination of the anti-CD30–auristatin-E antibody-drug conjugate SGN-35 with chemotherapy improves antitumor activity in Hodgkin lymphoma**

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Despite improvements in long term survival rates of patients with Hodgkin lymphoma (HL) treated with combined modality therapy (abbreviated chemotherapy and limited irradiation), new strategies to further improve outcome and reduce therapy-induced complications are needed. The antibody-drug conjugate (ADC) SGN-35 consists of the tubulin inhibitor monomethyl auristatin E (MMAE) conjugated to the anti-CD30 monoclonal antibody cAC10. This ADC potently interferes with the growth of CD30 positive hematologic tumors, including HL and anaplastic large-cell lymphoma (ALCL). Here we report SGN-35 localized into tumor xenograft in mice and showed potent antitumor activity in models of HL either alone or in combination. When combined with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine), or gemcitabine, the antitumor activity was markedly better than was observed with either SGN-35 or chemotherapy only regimens. Combination treatment was not associated with alterations in CD30 expression on tumors. Improved antitumor activity was also observed in high tumor burden models, indicating that combining SGN-35 with chemotherapeutic agents such as gemcitabine may be advantageous for the treatment of patients with relapsed or refractory HL.

**524** POSTER

**New biomolecule for cancer therapy**

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**Background:** Immune system plays a crucial role in the transformed cell elimination at early stage of tumorigenesis. This tumoral mechanism of surveillance requires different kind of cytotoxic cells notably Natural Killer cells (NK). NK cells activation depends on a balance between activatory and inhibitory signals. These signals are the result of a set interactions between ligand expressed by target cells and receptor on NK cells surface. Currently, one of the most studied NK cells activators receptor is NKG2D (NK Group 2-D receptor) which is expressed constitutively on NK cells surface. This receptor binds to several ligands including MICA (MHC Class I Chain-related protein A) which is expressed by human transformed cells. It has been demonstrated that tumor cells transfected to overexpress MICA are no more able to grow in vivo due to NK and CD8+ T cells response.
We designed new biomolecules with the aim to restore presence of MICA at tumor cell surface to mime the interaction between NKG2D receptor and MICA in order to induce a specific lysis of tumor cells induced by NK cells. These recombinants biomolecules are composed of MICA and scFv (single chain fragment variable) which recognize tumor associated antigen (TAA), linked by a Fc fragment of a human IgG1.
**Material and Methods:** The recombinant proteins were expressed in eukaryotic mammalian cells, then purified by affinity chromatography. The integrity of these protein was evaluated by SDS-PAGE and their functionality analyzed by flow cytometry and chromium release cytotoxic assay.
**Results:** In vitro experiments showed that purified recombinant proteins specifically bind to tumor cells expressing relevant TAA which was also confirmed by flow cytometry. More importantly, these recombinant proteins could induce the killing of tumor cells when incubated in presence of NK cells (Figure 1A, 1B).
**Conclusion:** We designed and produced recombinant proteins able to specifically target different tumor cells and induce their killing by NK cells in vitro. These biomolecules are now under investigation for an in vivo therapy.

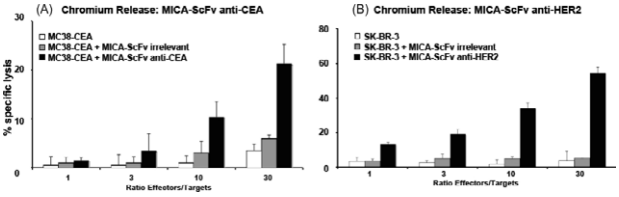


Figure 1. (A) NK-92 cell culture analysis for cytotoxicity against MC38-CEA cells (mouse colon carcinoma cell lines which express the associated tumor antigen CEA). The target cells were incubated in RPMI medium alone (white bars), with an irrelevant molecule (grey bars) or with the biomolecule of interest, MICA-ScFv anti-CEA (black bars). (B) NK-92 cell culture analysis for cytotoxicity against SK-BR-3 cells (human breast carcinoma cell lines which express the associated tumor antigen HER-2). The target cells were incubated in RPMI medium alone (white bars), with an irrelevant molecule (grey bars) or with the biomolecule of interest, MICA-ScFv anti-CEA (black bars).

**525** POSTER

**Preclinical evaluation of SAR566658 (huDS6-DM4) in mice bearing human tumor xenografts of breast, ovarian, lung, cervical and pancreatic cancer**

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SAR566658 (huDS6-DM4) consists of the humanized monoclonal antibody, huDS6, with DM4, a potent cytotoxic maytansinoid, attached, and targets solid tumors such as ovarian, breast, cervical, lung and pancreatic carcinomas. The DS6 antibody was raised in mice using human serous ovarian cancer ascites as the immunogen and recognizes an O-linked tumor-associated sialoglycotope on Muc1. The DS6 antibody was humanized using resurfacing technology that preserved the binding affinity (0.3 nM) of the antibody and the cytotoxic activity (1 nM) and selectivity of the conjugate for antigen-positive tumor cells.
SAR566658 is active in a dose-dependent manner against several human xenograft solid tumors in immunodeficient mice. For example, mice bearing tumors (142 mg) of the human breast tumor cell line, UISO BCA-1, a tumor model refractory to standard chemotherapeutic agents, were treated twice per day on days 17 and 21 with IV doses of SAR566658. At the highest non-toxic dose (HNTD) of 20.8 mg/kg/injection (total dose: 83.2 mg/kg), SAR566658 induced a body weight loss of 11.4% and was considered highly active with 100% complete tumor regressions (CR), a 5.2 log cell kill lck = tumor growth delay/3.32 x tumor doubling time) and 40% of the mice tumor free at the end of study (day 141). Lower doses (51.6 and 32 mg/kg total dose) were also highly active, causing 100% and 80% CR with a 5.3 and 3.3 lck, respectively. The lowest active dose was 19.6 mg/kg total dose (1.9 lck). On the other hand, treatment with either the huDS6 antibody (total dose: 51.6 mg/kg) or free DM4 (1 mg/kg; equivalent DM4 dose to 51.6 mg/kg SAR566658) showed no anti-tumor activity. In addition, standard chemotherapeutics doxorubicin and cyclophosphamide (highest dose tested 9.0 mg/kg/injection and 300 mg/kg/injection, respectively) were inactive against the UISO BCA-1 xenografts, while 5-fluorouracil showed modest activity (2.0 lck) at its HNTD (90 mg/kg/injection). Dose-dependent activity with SAR566658 was also observed in other antigen-positive human xenografts with 100% CR achieved in breast (MX-1 at 26.8–69.6 mg/kg), cervical (WISH at 3.9−46 mg/kg), ovarian (OVCAR5 at 16−46 mg/kg), pancreatic (Capan-2 at 5.2−23 mg/kg) and lung (NCI-H460 at 43.4 mg/kg) models. The dependence of SAR566658 activity on binding to target antigen was demonstrated in the human ovarian OVCAR5 model where the anti-tumor activity of SAR566658 was inhibited by pretreatment (2 hours earlier) of mice with excess huDS6 antibody. SAR566658 was not only able to elicit CR and tumor-free survivors in mice with advanced tumors (100–200 mg at the start of treatment), but also in mice with bulky tumors (~500 mg) arising from recurrent disease. The robust activity of SAR566658 in preclinical models provides a strong rationale for its clinical development.