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Allogeneic MHC Class I conjugated to antitumor antibody can induce regression of syngenic tumor grafted in vivo

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Background: To redirect a T-cell response against tumor cells, a bifunctional protein consisting of an anti-tumor antibody fragment linked to an allogeneic Major Histo-Compatibility class I (MHC I) molecule has been evaluated.
Materials and Methods: To realize the proof of concept, a recombinant allogeneic MHC I has been first chemically linked to a Fab′ fragments from monoclonal antibody (mAb) specific for the carcinoembryonic antigen (CEA). A recombinant protein composed of a single chain MHC class I molecule fused to two different scFv (single chain fragment variable) specific for CEA or for the receptor HER2 has been further designed. A murine IgG1 fragment Fc was used as linker between MHC I and scfv. This fragment allows the dimerization between these both entities. Recombinant purified proteins expressed in mammalians cells have been analysed by SDS PAGE, flow cytometry and chromium release assay. In vivo, the therapeutic potential of this approach has been tested in immunocompetent CEA transgenic mice grafted with a murine colon carcinoma cell line expressing CEA (MC38-CEA+) (tumor syngenic model).
Results: SDS PAGE analysis and exclusion chromatography have shown that we are able to product pure chemical and recombinant conjugates. A specific coating of allogeneic MHC I on murine cell line with both chemical and recombinant conjugates have been demonstrated by flow cytometry analysis. Finally, the chromium release assay has shown that the potent killing ability of CD8 T lymphocytes could be redirected against different tumor cells by the two types of conjugates. It has so proven the in vitro conjugate activity. Systemic injection of chemical anti-CEA/allogeneic MHC I conjugate in the CEA Transgenic mice bearing MC38 CEA+ tumors completely abolish tumor growth in 60% of mice. Moreover, the mice treated are protected against a new graft of MC38-CEA+ cell line.
Conclusions: In vivo, the results obtained with the chemical conjugate show that a physiologic T-cell allogeneic response in immunocompetent mice can be redirected against tumor cells by the use of anti-tumor antibody/allogeneic MHC I conjugates. In vitro efficiency of our recombinant proteins is confirmed, and in vivo the therapeutic used of these proteins is still investigated. These results open the way to the development of immunotherapy strategies based on antibody-mediated targeting of allogeneic MHC I.

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Gemcitabine versus combined antibodies against EGF receptors in pancreatic cancer: preclinical findings for clinical development

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Background: Pancreatic carcinoma remains a treatment-refractory cancer with a poor prognosis. Gemcitabine remains the gold standard treatment in this disease but lacks of long-term efficacy, namely in metastatic setting. EGFR and HER2 have been reported to be both dysregulated in this cancer and associated with a worse prognosis. In the present study, we compared combined anti-EGFR/anti-HER2 antibodies or gemcitabine in first and second line treatments in terms of xenograft progression, mice survival, and histological tumor response after treatments. In addition, we assessed the predictive value of K-ras status.
Materials and Methods: Nude mice bearing xenografts of BxPC-3, MiaPaCa-2, or Capan-1 human pancreatic carcinoma cell lines were injected twice a week with combined anti-EGFR (cetuximab) and anti-HER2 (trastuzumab) mAbs in (ratio 1/1, 2 mg/kg) or gemcitabine (150 mg/kg). In second line i.e. progression under gemcitabine treatment, animals received mAbs combination. Kras status was analyzed by direct sequencing.
Results: In all cell lines used, mice survival was significantly longer in MAbs group compared to control and gemcitabine groups ($p < 0.0001$). In addition, complete remission and cured mice with a long follow-up were only observed in the MAbs group. In second line, tumors treated with MAbs showed significant tumor regressions in all mice compared to mice treated with gemcitabine. Similarly, mice survival in the three pancreatic models was significantly longer after introducing MAbs than treated only with gemcitabine ($p = 0.008$ for BxPC-3, $p = 0.05$ for MiaPaCa-2 and $p = 0.002$ for Capan-1 model). Therapeutic benefit of combined cetuximab and trastuzumab was observed whatever Kras status of cell lines. Immunohistochemistry analyses showed a decrease of EGFR expression and inhibition of proliferation index (Ki67 and mitosis) after first-line of MAbs. An increase of KI67 (up to 30%) and pAKT (30%) was observed in tumors presenting resistance to MAbs.
Conclusions: This pre-clinical study demonstrated a significant improvement of survival and tumor regression in mice treated with combined anti-EGFR and anti-HER2 antibodies in first and second line of treatment whatever the status of K ras. Pathological and molecular experiments are still on-going to better understand intrinsic mechanisms.

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Vascular endothelial growth factor receptor-1 (VEGFR1) immunoreactivity in human renal carcinoma

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While multi-tyrosine kinase receptor inhibitors whose targets include vascular endothelial growth factor receptors (VEGFRs) have been approved for the treatment of renal cell carcinoma, the specific receptors contributing towards cancer progression have not been identified. VEGFRs can play an important role in cancer pathogenesis with regard to tumor vascularity and metastasis. RCC is a well vascularized tumor that can metastasize to the lung and bones. Compared with normal kidney, VEGFR1 mRNA is elevated in RCC. Furthermore, VEGFR1 mRNA is reportedly higher in renal cell carcinoma (RCC) samples than in many other tumor types.
In the present study, to follow up on reports of elevated mRNA in RCC, VEGFR-1 protein expression was evaluated in formalin fixed paraffin embedded human renal carcinoma tissue arrays utilizing a murine monoclonal antibody FB6 specific for VEGFR1. We have previously demonstrated VEGFR1 expression on blood vessels and tumor cells in tumor tissue specimens by immunohistochmistry (IHC) using FB6. VEGFR1 IHC was performed on RCC tissue from 69 individual patients and 3 normal kidney tissues. Normal kidney showed strong staining on blood vessels throughout the glomerulus and outside the glomerulus. In the RCC samples, blood vessels in all specimens were also clearly stained by FB6 antibody. VEGFR1 immunoreactivity was associated with the inner lining of blood vessels, supporting its expression by endothelial cells. 67% of RCC specimens contained VEGFR1 positive tumor cells localized to the plasma membrane and/or the cytoplasm. Importantly, in a semiquantitative evaluation, 75–100% of tumor cells in 30% of RCC samples demonstrated VEGFR1 immunoreactivity. In previous analyses of breast, colorectal, pancreatic, prostate, head and neck, NSCLC and ovarian cancer tissue arrays, the highest percent of tumor samples to achieve this level of VEGFR1 expression was breast cancer at 15%. Higher expression of VEGFR1 by renal carcinoma cells may support this cancer type for anti-VEGFR1 targeted therapy.

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Location. . . location. . . location: hitting the functional epitope within the target is essential for anti-cancer antibody therapeutics

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The challenge in designing effective target-specific cancer drugs is the identification of targets that are important in the progression and initiation of cancer. Whether the target is a validated in cancer, the specific region or location targeted within the molecule is crucial to the design and development of effective drugs. This is especially true for monoclonal antibody therapies where multiple antibodies generated to different epitopes of the same target can potentially lead to very different outcomes. The ability to parse out functionally effective epitopes from ineffective epitopes within a specific target determines the generation of successful drugs. The FunctionFIRSTTM screening platform inherently discovers functional antibodies against validated targets as well as novel cancer associated molecules while at the same time identifying efficacious epitopes. Using this platform, ARIUS has generated effective antibodies against numerous validated cancer targets for which there are currently no therapeutic antibodies in clinical development. To illustrate the importance of epitopes on a target, we have generated several antibodies that target the novel cancer antigen, TROP-2. While a pair of functional antibodies demonstrates good in vitro activity and binding to cancer cell lines, differ in their in vivo efficacy. AR47A6.4.2 has greater tumor growth inhibition (TGI) compared with the other antibody in xenografts of human PL45 pancreatic cancer (100% compared to 50% TGI, respectively) and MDA-MB-231 breast cancer (92% compared to 48.3% TGI, respectively). Epitope mapping of these antibodies revealed that they bind to non-identical

overlapping regions of TROP-2 demonstrating the importance of localizing the correct area within a target molecule. To further test this hypothesis, anti-TROP-2 antibodies were selected from a pool of antibodies shown to be non-functional in cell killing by the FunctionFIRSTTM screen. When these were tested in vivo, the non-functional anti-TROP-2 antibodies did not display tumor growth inhibition in the PL45 xenograft model, while the efficacy of AR47A6.4.2, currently being advanced to clinical trials, was confirmed. Thus, targeting the functional epitopes within a target is necessary to designing new therapies. The ability to screen monoclonal antibodies that to target relevant epitopes is a powerful tool for increasing efficiency in discovering and developing novel and effective drugs.

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Discovery of the 6F4 anti-tumor antibody targeting the tight junction molecule JAM-A. 2. Target expression on human tumors and in vitro and in vivo anti-cancer activity

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Biotherapeutic drugs in oncology are generating a great enthusiasm and tremendous amount of effort is made to identify new targets for the development of news therapeutics. Functional approaches using tumor cells as immunogens have been performed in order to select on the basis of their function, molecules that, targeted by monoclonal antibodies (Mabs), would induce inhibition of cell proliferation both in vitro and in vivo.
After immunization of mice with MCF-7 breast cancer cells, we generated a series of Mabs able to block MCF-7 cell proliferation in vitro. These Mabs were further tested in vivo for their ability to induce MCF-7 tumor regression in engrafted nude mice. Interestingly, one Mab named 6F4 was able to completely inhibit tumor growth in mice after i.p. treatment with 1 mg dose twice a week. A proteomic analysis using 6F4-tagged beads coupled to a MS analysis indicated that 6F4 Mab specifically recognized the human Junctional Adhesion Molecule A (JAM-A). The activity of 6F4 Mab was also observed after injection of 1 mg of the Mab twice a week, to mice xenografted with human epidermoid squamous carcinoma A431 cells.
Immunohistochemistry analysis on a panel of human tissues from normal and tumor origin revealed that the JAM-A molecule was strongly overexpressed on tumor tissues compared to normal ones. In order to get inside the mechanism of action of 6F4 Mab, ex vivo analysis was performed on MCF-7-xenografted mice. We observed a dose-dependent inhibition of cell proliferation together with a disappearance of JAM-A from the cell surface, in perfect agreement with the results obtained with cells in vitro. These results indicate that the 6F4 anti-JAM-A Mab is able to induce tumor growth inhibition and therefore suggest that JAM-A is a potential novel target in oncology. Further experiments are needed to better characterize the mechanism of action of the lead antibody. The results also demonstrate that a functional approach coupled to proteomic analysis can be successful to identify new antibody target molecules that lead to promising new antibody-based therapies against cancers.

Radiation interactive agents

545 POSTER

Enhancement of cell motility with radiation-induced VEGF in glioma

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Background: Glioblastoma multiforme (GBM) is among the most lethal of all human tumors, with poor survival and frequent local recurrences after treatment. The mechanism accounting for such a recurrence pattern is unclear. It has classically been regarded as a local recurrence of treatment resistant cells. However, accumulating evidence suggests that a second mechanism may exist involving the migration of tumor cells or tumor stem cells from regions of the brain that received minimal RT. VEGF family members are well known as active mitogens and are up-regulated after RT. Here, we examine the effect of irradiation (IR)-induced VEGF on glioma cell motility.
Material and Methods: The U251 and LN18 human GBM cell lines were used to generate conditioned medium (CD). At 72 hours after various dose of IR, the supernatant of culture dishes were harvested and used as IR-CD. Actinomycin D (Act D) was used as a transcription inhibitor. ELISA was used to quantity the VEGF protein in conditioned medium. The expression level for VEGF mRNA transcripts was detected by RT-PCR. In vitro motility assay was done with chamber coated with/without Matrigel and CD as a cell motility enhancer. The VEGF antibody was used for neutralization of VEGF bioactivity in conditioned medium.
Results: ELISA showed the VEGF was increased in CD at 72 hours after IR (98.504±0.098pg/1000cells with 2 Gy v.s. 0.034±0.003pg/1000cell in control). RT-PCR revealed an increase of VEGF mRNA after IR, and the effect was mitigated by pre-RT exposure to Act D (432±53% and 75±14% of control, respectively). Cell motility (migration and invasion) was enhanced with the addition of IR-CD (174.9±11.4% and 334.2±46% of control, respectively). The enhanced cell motility measured with the addition of IR-CD was negated with the addition VEGF antibody to IR-CD (110.3±12.0% and 105.7±14.0% of control, respectively).
Conclusions: These results indicate that cell motility can be enhanced by conditioned medium from irradiated cells in vitro and suggest that this effect involves radiation-induced VEGF leading to an increase in glioma cell motility.

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GI261 brain tumor cells: responses to single or fractionated x-irradiation with the avβ3 integrin thyroxine receptor antagonist TETRAC (tetraiodothyroacetic acid)

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Background: The membrane integrin avβ3 has been shown to contain a receptor for thyroid hormone. TETRAC-a product of L-thyroxine, inhibits the proliferative/angiogenic effects of L-thyroxine and L-thyronine (T3, T4) initiated at the integrin receptor near or at the Arg-Gly-Asp (RGD) recognition site.. Since targeting the avβ3 integrin with cilengitide, an Arg-Gly-Glu tripeptide, results in increased cellular radiosensitivity, (Albert et al Int. J. Radiat. Oncol. Biol. Phys. 2006: 65: 1536–1543), we tested TETRAC as a radiosensitizing agent.
Methods and Materials: Glioma (GL261) cells were grown as exponential or plateau phase cultures and treated with 2 μM TETRAC for 1 h at 37ºC prior to exposure to 250 kVp x-rays. After irradiation, cells were removed from treatment dishes, counted and then re-plated in fresh medium (without TETRAC) for estimation of effect of TETRAC on immediate survival. Results were analyzed using the linear-quadratic (LQ) formalism. In other experiments, the time-dependent effects of TETRAC on sublethal and potentially lethal damage repair (SLDR and PLDR) were determined.
Results: LQ parameters for control cells were: a = 0.360 Gy-1 and β = 0.094 Gy-2. 2 Gy survival was 33.4%. For cells treated with TETRAC, LQ parameters were: a = 0.921 Gy-1 while β was zero. Survival at 2 Gy was 15.5%. The 2 Gy dose ratio yielded an enhancement factor of 2.2. TETRAC also reduced SLDR in exponential cells by 62%. In control exponential cells, there was little PLDR expression with an increase in survival of 1.5-fold seen at 8 h post-irradiation. TETRAC, however, completely removed PLDR expression in exponential cells. Control plateau phase cells reached an increase in survival of 4-fold 8 h post-irradiation. TETRAC decreased the expression of this PLDR by 75%.
Conclusions: TETRAC, which blocks the avβ3 integrin receptor from T3/T4 effects resulted in both radiosensitization and inhibition of SLDR and PLDR. This may involve occlusion of the RGD site which binds radioprotective FGF-2 and VEGF.

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Effect of the extract of *Taraxacum officinale* on inflammation induced by anti-cancer treatment

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Purpose: Mucositis is a major complication associated with anti-cancer treatment. Inflammation is the main process of mucositis from both chemotherapy and radiotherapy. Many herbs including *Taraxacum officinale* (TO) have anti-inflammatory effect and we tried to evaluate that the extract of TO could reduce the inflammation from anti-cancer treatment.
Materials and Methods: Female Wistar rats for methotrexate (MTX) induced enteritis were orally administered with 3 different concentration of the TO extracts or saline for 12 days from 1 week before MTX injection and then MTX (2.5 mg/kg) were subcutaneously injected for 3 days to induce intestinal musocitis. The histologic severity of intestinal damage from MTX treatment was assessed semiquantitatively 5 days after injection. Rats for radiation induced proctitis received 17.5Gy of radiation on rectal mucosa. They were fed TO or saline for 7 days prior to radiation and continued for 10 days after irradiation. Rats were sacrificed 10 days and 6 weeks after irradiation for histologic evaluation of acute and chronic phase. For in vitro study, RAW 264.7 cells were treated with TO for 24 hours and then irradiated. We collected culture supernatant 48 hours after irradiation and