responses and one partial response in the 7 animals treated at 10 mg/kg. IMGN388 also demonstrated efficacy against established human tumors of colon (HT-29), large cell lung (H460), pancreatic (AsPC-1), ovarian (A2780, SKOV-3), and breast (MDA-MB-231.OT.F2) carcinomas in nude rat models. Additionally, IMGN388 has been found to inhibit angiogenesis using an in vivo model of basic fibroblast growth factor-induced angiogenesis in nude rats. Thus, the anti-tumor effects of IMGN388 can be attributed to two distinct mechanisms of action: direct tumor-cell killing and anti angiogenic activity.
Conclusion: The broad expression of the target integrin among solid tumors and the observed anti-tumor efficacy of IMGN388 in xenograft models of pancreatic, colon, lung, breast, and ovarian carcinomas in rats support the clinical evaluation of IMGN388 for the treatment of solid tumors.

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Use the humanized anti-EGFR MAb (nimotuzumab) and radiotherapy for the treatment of high grade glioma patients

T. Crombet Ramos[1], J. Figueredo[2], M.T. Salomón Cárdenas[3], J.C. Selva Infante[4], C. Toledo Jiménez[5], J. Vaquer[6], N. Quintanal[7], S. Salva Camaño[8], R. Odelín Tablada[9], J.J. Marinello[10]. *[1]Center of Molecular Immunology, Clinical Direction, Havana, Cuba; [2]Center for Medical and Surgical Research, Neurosurgery, Havana, Cuba; [3]Calixto García, Neurosurgery, Havana, Cuba; [4]Lucía Iñiguez, Neurosurgery, Holguín, Cuba; [5]Maria Curie, Neurosurgery, Camaguey, Cuba; [6]Arnaldo Milián, Neurosurgery, Villa Clara, Cuba; [7]Luis Díaz Soto, Neurosurgery, Havana, Cuba; [8]Hermanos Ameijeiras, Neurosurgery, Havana, Cuba; [9]Saturnino Lora, Neurosurgery, Santiago de Cuba, Cuba; [10]National Institute of Oncology, Radiotherapy, Havana, Cuba*

Background: The incidence of brain tumors is worldwide increasing and despite advances in neurosurgery and radiotherapy, limited progress has been made in the treatment of patients with high-grade gliomas. Amplification and rearrangement of the Epidermal Growth Factor Receptor (EGFR) have been found in primary high-grade astrocytomas. For primary brain tumors, over-expression of EGFR has been associated with poor survival due to growth advantages. Nimotuzumab is a humanized monoclonal antibody that recognizes EGFR with high affinity, inhibiting tyrosine kinase activation.
Material and Methods: A Phase II/III clinical trial was conducted to evaluate the efficacy and safety of nimotuzumab in combination with radiotherapy in newly diagnosed high-grade glioma patients. It was a multicentric, controlled, double blinded trial where 80 patients bearing high grade glioma were randomized to receive radiotherapy and nimotuzumab or irradiation plus a placebo. Patients received 6 weekly infusions of the placebo or nimotuzumab (200 mg) while they were receiving radiotherapy. After irradiation, patients received a maintenance dose of the investigational drug, every 21 days until completing a year of treatment.
Results: So far, 65 patients have been enrolled, 30 patients bearing glioblastoma and 35 bearing anaplastic astrocytomas. Fifteen additional anaplastic astrocytoma patients should be enrolled to finish the trial. All patients had surgery (biopsy, partial or total resection) before the inclusion in the trial. Both groups were very well balanced in relation to the factors that predict the outcome of the disease: Karnofsky index, previous surgery and age. Since the enrollment of the glioblastoma stratum is finished, a preliminary evaluation of safety and survival was done. The antibody was very well tolerated. Adverse events were more frequent in the placebo arm as compared to the nimotuzumab arm. The antibody did not provoke skin rash or allergic reactions. A preliminary survival analysis was done for all subjects bearing glioblastoma that received curative intent radiotherapy. The mean and median survival time for the patients treated with nimotuzumab plus radiotherapy was 14.31 and 16.43 months, respectively, while the mean and median survival time for the placebo arm was 8.67 and 10.49 months. The median survival time is similar to the one reached in the previous single arm study in patients bearing glioblastoma treated with nimotuzumab and radiotherapy (17.43 months) and compares favorably with the overall survival after irradiation and temozolomide (14.6 months). The evaluation of human anti-humanized antibody response (HAHA) is ongoing.
Conclusions: For the subgroup of glioblastoma multiforme patients, nimotuzumab combined with radiotherapy was safe and showed a trend toward a survival benefit as compared to the placebo arm.

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Junctional complexes as a factor limiting the extravascular penetration of trastuzumab

K.E. Lindquist[1], J.T. Sy[1], A.H. Kyle[1], J.H. Baker[1], A.I. Minchinton[1]. *[1]British Columbia Cancer Research Centre, Medical Biophysics, Vancouver, BC, Canada*

The tumor microenvironment presents many barriers to drug penetration, including abnormal microvessel structure and function, deficient or absent lymphatics and variable extracellular matrix composition. Previously, using immunohistochemical mapping of MDA-435/LCC6^{HER2} xenografts we found the extravascular distribution of trastuzumab (generic, Herceptin®), to be incomplete and highly heterogeneous. To characterize properties of the tumor microenvironment that govern trastuzumab penetration, we extended these studies using HER2 over-expressing MCF-7 breast cancer cells (MCF-7^{HER2}) and a tight junction marker, ZO-1. Additionally, we used multilayered cell cultures (MCCs) in conjunction with transmission electron microscopy (TEM) to assess trastuzumab penetration through MCF-7^{HER2} tumor cells in vitro.
In mapping studies, mice bearing MCF-7^{HER2} tumors were given single doses of 4 mg/kg trastuzumab with tumor harvest at various time points thereafter; bound trastuzumab was imaged in tumor cryosections using fluorescent anti-human secondary antibodies. Combinations of additional markers, including HER2, 5-bromo-2-deoxyuridine, CD31, DioC7, and ZO-1 were also mapped on the same tumor sections. MCF-7^{HER2} MCCs were exposed from both sides to 60ug/mL trastuzumab for 1−24 h before drug removal, washing, and freezing. MCCs were cryosectioned and immunostained for trastuzumab, HER2, and ZO-1. For TEM studies untreated MCCs were fixed in gluteraldehyde, treated with osmium tetroxide and embedded in epoxy resin; ultra-thin 60 nm sections were imaged.
Similar to the MDA-435/LCC6^{HER2} model, 4 mg/kg trastuzumab did not saturate MCF-7^{HER2} tumors even after 72 h following administration. Trastuzumab exposure to both sides of MCF-7^{HER2} MCCs revealed an interesting phenomena wherein trastuzmab penetrated from only one surface of the discoid culture, despite ubiquitous HER2 expression. Staining for ZO-1 revealed the presence of continuous tight junctions along the surface of the culture disallowing trastuzumab penetration. TEM images confirmed the existence of tight junctions along the surface of MCF-7 MCCs. This suggests paracellular transport is required for trastuzumab penetration and implicates the need for structurally aberrant vasculature within tumors to facilitate the extravascular distribution of trastuzumab. This observation warrants further investigation into the junctional complexes of tumor tissue and endothelium as a factor limiting the penetration of anti-cancer agents.

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A chimerized anti-CD4 monoclonal antibody for the treatment of T cell lymphomas acts through activation of membrane acid sphingomyelinase leading to increased ceramide release and CD4/ZAP-70 protein redistribution in membrane rafts

M. Chentouf[1], M. Rigo[1], A. Pèlegrin[1], T. Chardès[1]. *[1]Institut de Recherche en Cancérologie de Montpellier, INSERM Unit 896/Université de Montpellier 1, Montpellier, France*

Background: recombinant IgG1 antibody 13B8.2 (rIgG1 13B8.2) binds to the CDR3-like loop on the D1 domain of CD4, and both inhibits proliferation and induces complement- and antibody-dependent cell cytotoxicity of T lymphoma cells. The biological effects of rIgG1 13B8.2 are partly due to signals that prevent NF-κB nuclear translocation, but precise mechanisms of action, particularly at the level of membrane proximal-signalling, remains obscure.
Materials and Methods: upon crosslinking of Jurkat T lymphoma cells with rIgG1 13B8.2, membrane rafts were extracted using Brij98 as detergent at 37ºC and subsequently separated by sucrose gradient centrifugation. Protein analysis was performed by western blot using appropriate antibodies. Lipid composition was measured by using Amplex red kits for cholesterol and acid sphingomyelinase (Invitrogen), and thin-layer chromatography.
Results: rIgG1 13B8.2 was found to induce an accumulation/retention of the CD4 molecule inside Brij 98 detergent-resistant membrane rafts, together with recruitment of TCR, CD3, p56 Lck, Lyn and Syk p70 kinases, LAT and Cbp/PAG adaptor proteins, and PKCtheta, but excluded ZAP-70 and its downstream targets SLP-76, PLCgamma1, and Vav-1. Analysis of key upstream events such as ZAP-70 phosphorylation showed that modulation of Tyr292 and Tyr319 phosphorylation occurred concomitantly with 13B8.2-induced ZAP-70 exclusion from the membrane rafts. rIgG1 13B8.2 did not affect membrane cholesterol but increased ceramide synthesis in membrane raft, in correlation with enhanced acid