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**Señor**
**DIRECTOR DIVISIÓN DE NUEVAS CREACIONES**
**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**
E. S. D.

REF: Expediente No. **07 12528**– solicitud de patente de invención a favor de **GENENTECH, INC. FOUNDATION.** Título **ANTAGONISTAS HUMANIZADOS ANTI-CMET**

**RESPUESTA ARTICULO 46**

**JUAN PABLO CADENA SARMIENTO**, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante me permito dar respuesta al oficio número 1051 relacionado con la patentabilidad de la presente invención y notificado por fijación en lista el 26 de enero de 2012 en los siguientes términos:.

- ✓ Adjunto copia del artículo solicitado en virtud del artículo 46 de la Decisión 486.

Del Señor Director

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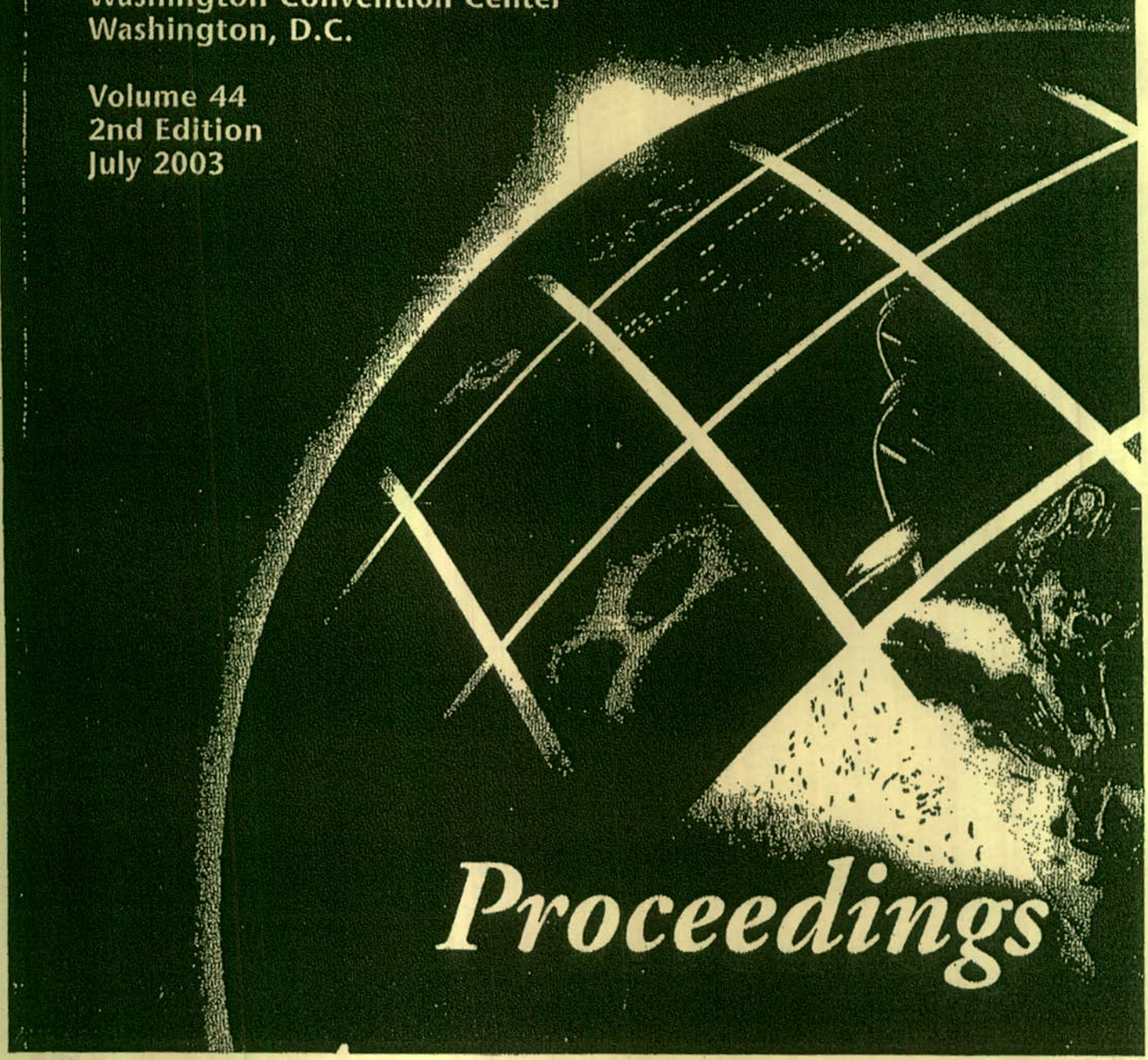

# Proceedings

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sponses in several patients. To reduce the immunogenicity of CC49 in patients, a humanized CC49 (HuCC49) and its variant HuCC49V10 (V10) were developed. HuCC49 was generated by conventional grafting of complementarity determining regions (CDRs), while the variant was developed by grafting only the specificity determining residues (SDRs) of CC49 onto the VL and VH frameworks of the human antibodies LEN and 21/28'CL. Although V10 has reduced reactivity to sera from patients who were previously administered murine CC49 in clinical trials, its antigen-binding affinity is 2-3 fold lower than that of HuCC49. This report describes generation and characterization of variants of V10, with higher antigen binding affinity and lower sera reactivity. To this end, in vitro affinity maturation of V10 was carried out using phage display technique. A limited library of mutant Fabs was generated by replacing some of the SDRs of the light- and heavy-chain CDRs with all possible residues located at the corresponding positions in human antibodies. The library was enriched in Fabs that have high binding affinity to the TAG-72 antigen by several rounds of panning. The clones encoding the best binders were expressed in insect cells as whole antibodies that were purified and characterized. Competition radioimmunoassay and surface plasmon resonance (SPR) measurements showed that two of the isolates, V14 and V15, have a higher binding affinity than that of V10. More importantly, reactivity of the variants and the parental antibody to the anti-variable region antibodies in sera from patients in phase I clinical trial of murine CC49 by SPR analysis showed that V14 and V15 have much lower human sera reactivity than that of V10. The two isolates, V14 and V15, which show significantly higher antigen-binding reactivity and much lower sera reactivity than the parental V10 antibody, are potentially excellent reagents for clinical applications.

**#5604 In vitro and in vivo activity of fully-human monoclonal antibodies to c-Met protein tyrosine kinase.** Phillip A. Morton, William D. Joy, Christine P. Bono, J. Alan Arbuckle, Michelle L. Evans, Michiko S. Huynh, Larry E. Kahn, Herbert A. Runnels, Mark O. Palmier, Mark A. Moffat, J. J. Shieb, Duo Sun, Vicky W. Holway, Joseph K. Welply, Gerald F. Casperson, Christine E. Smith, Duncan Cochrane, Debbie Marston, and Andy Roberts. *Pharmacia Corporation, St. Louis, MO and Cambridge Antibody Technology, Cambridgeshire, UK.*

Increased expression and activity of c-Met protein tyrosine kinase is associated with many solid human cancers and blockade of c-Met activity represents a promising targeted approach to human cancer therapy. We have sought to identify therapeutic antibodies against c-Met which block ligand binding and kinase activation. Screening of phage display libraries identified fifty single-chain fragment variable (scFv) proteins that prevent binding of hepatocyte growth factor (HGF), with IC50 <50 nM. A subset of the ligand-blocking scFv against c-Met were then engineered and expressed as fully-human IgG1 for additional in vitro and in vivo studies. IgG1 isotype monoclonal antibody (mAb) exhibited high affinity for c-Met in BIAcore assay (range = 0.8 - 55 nM) and effectively competed for HGF binding to c-Met extracellular domain (ELISA; range = 0.45 to 2.9 nM). However, these antibodies stimulated c-Met tyrosine kinase phosphorylation and enhanced cell proliferation and scattering at ~10 nM, while they only weakly inhibited HGF-dependent cell proliferation, scattering and migration at concentrations > 100 nM. Full-length agonist c-Met mAbs were observed to enhance the growth of S114 and H441 human xenografts expressing c-Met and autocrine HGF in nude mice. We attempted to minimize the partial agonist activity observed with full-length c-Met IgG by generation of Fab fragments. Fab fragments derived from c-Met mAb could still compete, albeit with lower affinity, for HGF binding to c-Met and did not induce c-Met tyrosine phosphorylation unless cross-linked by a secondary antibody. However, Fab fragments from c-Met IgG showed no enhanced ability to inhibit HGF-dependent cell proliferation, cell scattering, or cell migration beyond that observed with their full-length counterparts. In conclusion, affinity-matured monovalent versions of fully-human c-Met IgG and/or NK4 should be further considered as biological approaches to modulate c-Met activity in human cancers.

**#5606 Enhanced inhibition of tumor growth using targeted delivery of an antibody-endostatin fusion protein.** Seung-Uon Shin, Hyun-Mi Cho, Young-Sook Kang, Luisa Iruela-Arispe, Sherie L. Morrison, and Joseph D. Rosenblatt. *Sylvester Comprehensive Cancer Center, University of Miami School of Medicine, Miami, FL, Sook-Myung Women's University, Seoul, South Korea, and University of California, Los Angeles, Los Angeles, CA.*

Endostatin is a potent and specific antiangiogenic protein capable of inhibiting the growth of murine and xenotransplanted human tumors. Factors affecting the utility of endostatin in humans may be the short half-life of the protein, necessitating frequent dosing, and low concentration at the site of tumors. To increase efficacy, we have combined endostatin with the targeting specificity of an antitumor antibody by producing antibody-endostatin fusion proteins specific for the HER2/neu tumor antigen. We wish to use the targeting flexibility of antibodies to direct localization of endostatin to tumor. An anti-HER2/neu IgG3-CH3-endostatin fusion protein has been constructed, in which endostatin is fused to CH3 domain of anti-HER2/neu IgG3 antibody. In mice bearing CT26 or CT26 expressing HER2/neu (CT26-HER2) tumors and tumor free mice, endostatin is rapidly cleared from serum with a t1/2-beta elimination range of 38-225 min. Endostatin was rapidly degraded within 60 min (only 55% intact). In contrast, the anti-HER2/neu IgG3-CH3-endostatin fusion proteins showed a t1/2-beta of 2410-2640 min and increased stability (90% intact at 96 hours). 125I-labeled anti-HER2/neu IgG3-CH3-endostatin fusion protein specifically localized to CT26-HER2 tumors, relative to CT26. Using CT26-HER2 tumors implanted in BALB/c mice, we compared the effects on tumor growth of treatment with anti-HER2/neu IgG3-CH3-endostatin, anti-HER2/neu antibody or endostatin alone. Anti-HER2/neu IgG3-CH3-endostatin inhibited tumor growth more effectively than either endostatin or antibody alone. In addition, equimolar administration of anti-HER2/neu IgG3-CH3-endostatin to mice bearing both CT26 and CT26-HER2 showed preferential inhibition of CT26-HER2, compared to CT26 parental tumor contralaterally implanted within the same mice. Anti-HER2/neu IgG3-CH3-endostatin inhibited more effectively than endostatin, anti-HER2/neu IgG3 antibody, or the combination of antibody and endostatin. The longer half-life and serum stability of the anti-HER2/neu IgG3-CH3-endostatin fusion protein coupled with its ability to selectively target antigens expressed on tumors results in better suppression of angiogenesis. Antibody-endostatin fusion proteins may increase the potential efficacy of current antiangiogenic strategies, and may be superior to endostatin alone.

**#5607 Monoclonal antibodies recognizing human $gpnmb_{wt}$/$gpnmb_{sv}$ react with human high-grade gliomas (HGL).** Chien-Tsun Kuan, Carol J. Wikstrand, Kenji Wakiya, Gregory J. Riggins, and Darell D. Bigner. *Duke University Medical Center, Durham, NC.*

Targeting neoplastic cells of gliomas requires markers expressed on gliomas but not normal brain, preferably those on the cell surface, to be identified for cancer treatment. Immune-based targeted therapy is a promising approach to overcome the limited efficacy of conventional therapies for glioblastomas (GBM), the most common and most aggressive neuroectodermal neoplasms in adults. Human transmembrane *glycoprotein nmb* ($GPNMB_{wt}$) and a splice variant form ($GPNMB_{sv}$) leading to an in-frame insertion of 12 amino acids in the extracellular domain (ecd) have been identified by genetic and immunological means as potential glioblastoma tumor targets. In 50 patient samples, by quantitative PCR analysis, 4/6 (67 %) AA, 6/8 (75 %) AO, and 33/36 (92 %) GBM were positive for overall *gpnmb* transcripts ($gpnmb_{wt}$ plus $gpnmb_{sv}$). Splice variant gpnmbsv was detected in 2/6 (33 %) AA, 6/8 (75 %) AO, and 31/36 (86 %) GBM. In normal brain samples, little or no expression was noted for $gpnmb_{wt}$ and $gpnmb_{sv}$ mRNA. Specific rabbit polyvalent antiserum 2640 was readily produced by immunization with GPNMB ecd. We have recently characterized two $IgG_1$ anti-$GPNMB_{sv}$ MAbs, G11 and A3, isolated from fusions following immunization of mice with the non-glycosylated bacterially expressed $GPNMB_{sv}$ protein. Both MAbs readily detect the intact $GPNMB_{sv}$ protein in a Western Blot as expressed in HEK 293-$GPNMB_{sv}$ transfected cells; the staining pattern obtained is identical to that seen with the purified polyvalent rabbit anti-GPNMB serum 2640. In addition, both anti-$GPNMB_{sv}$ MAbs identify the same bands in the supernatant of recombinant $GPNMB_{sv}$-baculovirus infected-High Five insect cells, which secrete the intact $GPNMB_{sv}$ protein with slightly lower mass due