



# Biomarcadores en cáncer colorrectal: metaloproteinasa 7 en pacientes intervenidos y mutaciones tras progresión a terapias anti-EGFR en enfermedad metastásica

Alejandro Martínez Fernández

**ADVERTIMENT.** La consulta d'aquesta tesi queda condicionada a l'acceptació de les següents condicions d'ús: La difusió d'aquesta tesi per mitjà del servei TDX ([www.tdx.cat](http://www.tdx.cat)) i a través del Dipòsit Digital de la UB ([deposit.ub.edu](http://deposit.ub.edu)) ha estat autoritzada pels titulars dels drets de propietat intel·lectual únicament per a usos privats emmarcats en activitats d'investigació i docència. No s'autoritza la seva reproducció amb finalitats de lucre ni la seva difusió i posada a disposició des d'un lloc aliè al servei TDX ni al Dipòsit Digital de la UB. No s'autoritza la presentació del seu contingut en una finestra o marc aliè a TDX o al Dipòsit Digital de la UB (framing). Aquesta reserva de drets afecta tant al resum de presentació de la tesi com als seus continguts. En la utilització o cita de parts de la tesi és obligat indicar el nom de la persona autora.

**ADVERTENCIA.** La consulta de esta tesis queda condicionada a la aceptación de las siguientes condiciones de uso: La difusión de esta tesis por medio del servicio TDR ([www.tdx.cat](http://www.tdx.cat)) y a través del Repositorio Digital de la UB ([deposit.ub.edu](http://deposit.ub.edu)) ha sido autorizada por los titulares de los derechos de propiedad intelectual únicamente para usos privados enmarcados en actividades de investigación y docencia. No se autoriza su reproducción con finalidades de lucro ni su difusión y puesta a disposición desde un sitio ajeno al servicio TDR o al Repositorio Digital de la UB. No se autoriza la presentación de su contenido en una ventana o marco ajeno a TDR o al Repositorio Digital de la UB (framing). Esta reserva de derechos afecta tanto al resumen de presentación de la tesis como a sus contenidos. En la utilización o cita de partes de la tesis es obligado indicar el nombre de la persona autora.

**WARNING.** On having consulted this thesis you're accepting the following use conditions: Spreading this thesis by the TDX ([www.tdx.cat](http://www.tdx.cat)) service and by the UB Digital Repository ([deposit.ub.edu](http://deposit.ub.edu)) has been authorized by the titular of the intellectual property rights only for private uses placed in investigation and teaching activities. Reproduction with lucrative aims is not authorized nor its spreading and availability from a site foreign to the TDX service or to the UB Digital Repository. Introducing its content in a window or frame foreign to the TDX service or to the UB Digital Repository is not authorized (framing). Those rights affect to the presentation summary of the thesis as well as to its contents. In the using or citation of parts of the thesis it's obliged to indicate the name of the author.

**BIOMARCADORES EN CÁNCER COLORRECTAL:  
METALOPROTEINASA 7 EN PACIENTES INTERVENIDOS Y  
MUTACIONES TRAS PROGRESIÓN A TERAPIAS ANTI-EGFR EN  
ENFERMEDAD METASTÁSICA.**

Tesis presentada por  
**Alejandro Martínez Fernández**  
para aspirar a la titulación de Doctor en Medicina

Directores de la tesis:  
Dr. Joan Albanell Mestres  
Dra. Clara Montagut Viladot

Tutora de Tesis  
Dr. Pere Gascón Vilaplana

Programa de Oncología Molecular y Translacional  
Facultat de Medicina  
Universitat de Barcelona

Barcelona, 2014

## **I. BIBLIOGRAFÍA**



## BIBLIOGRAFÍA

Acar, A., Onan, A., Coskun, U., Uner, A., Bagriacik, U., Atalay, F., Unsal, D.K. & Guner, H. (2008). Clinical significance of serum MMP-2 and MMP-7 in patients with ovarian cancer. *Med Oncol*, **25**, 279-83.

Adachi, Y., Yamamoto, H., Itoh, F., Hinoda, Y., Okada, Y. & Imai, K. (1999). Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. *Gut*, **45**, 252-8.

Alessi, D.R. & Cohen, P. (1998). Mechanism of activation and function of protein kinase B. *Curr Opin Genet Dev*, **8**, 55-62.

Almendro, V., Ametller, E., Garcia-Recio, S., Collazo, O., Casas, I., Auge, J.M., Maurel, J. & Gascon, P. (2009). The role of MMP7 and its cross-talk with the FAS/FASL system during the acquisition of chemoresistance to oxaliplatin. *PLoS One*, **4**, e4728.

Amado, R.G., Wolf, M., Peeters, M., Van Cutsem, E., Siena, S., Freeman, D.J., Juan, T., Sikorski, R., Suggs, S., Radinsky, R., Patterson, S.D. & Chang, D.D. (2008). Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*, **26**, 1626-34.

Anastas, J.N. & Moon, R.T. (2012). WNT signalling pathways as therapeutic targets in cancer. *Nat Rev Cancer*, **13**, 11-26.

Andre, T., Boni, C., Mounedji-Boudiaf, L., Navarro, M., Tabernero, J., Hickish, T., Topham, C., Zaninelli, M., Clingan, P., Bridgewater, J., Tabah-Fisch, I. & de Gramont, A. (2004). Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med*, **350**, 2343-51.

Arteaga, C.L. (2001). The epidermal growth factor receptor: from mutant oncogene in nonhuman cancers to therapeutic target in human neoplasia. *J Clin Oncol*, **19**, 32S-40S.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J. & Clevers, H. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*, **449**, 1003-7.

Baselga, J., Pfister, D., Cooper, M.R., Cohen, R., Burtness, B., Bos, M., D'Andrea, G., Seidman, A., Norton, L., Gunnett, K., Falcey, J., Anderson, V., Waksal, H. & Mendelsohn, J. (2000). Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J Clin Oncol*, **18**, 904-14.

Bettegowda, C., Sausen, M., Leary, R.J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B.R., Wang, H., Luber, B., Alani, R.M., Antonarakis, E.S., Azad, N.S., Bardelli, A., Brem, H., Cameron, J.L., Lee, C.C., Fecher, L.A., Gallia, G.L., Gibbs, P., Le, D., Giuntoli, R.L., Goggins, M., Hogarty, M.D., Holdhoff, M., Hong, S.M., Jiao, Y., Juhl, H.H., Kim, J.J., Siravegna, G., Laheru, D.A., Lauricella, C., Lim, M., Lipson, E.J., Marie, S.K., Netto, G.J., Oliner, K.S., Olivi, A., Olsson, L., Riggins, G.J., Sartore-Bianchi, A., Schmidt, K., Shih I, M., Oba-Shinjo, S.M., Siena, S., Theodorescu, D., Tie, J., Harkins, T.T., Veronese, S., Wang, T.L., Weingart, J.D., Wolfgang, C.L., Wood, L.D., Xing, D., Hruban, R.H., Wu, J., Allen, P.J., Schmidt, C.M., Choti, M.A., Velculescu, V.E., Kinzler, K.W., Vogelstein, B., Papadopoulos, N. & Diaz, L.A., Jr. (2014). Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*, **6**, 224ra24.

Brabertz, T., Jung, A., Dag, S., Hlubek, F. & Kirchner, T. (1999). beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. *Am J Pathol*, **155**, 1033-8.

Broussard, E.K., Kim, R., Wiley, J.C., Marquez, J.P., Annis, J.E., Pritchard, D. & Disis, M.L. (2013). Identification of putative immunologic targets for colon cancer prevention based on conserved gene upregulation from preinvasive to malignant lesions. *Cancer Prev Res (Phila)*, **6**, 666-74.

Cancer-Genome-Atlas-Network. (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature*, **487**, 330-7.

Cappuzzo, F., Finocchiaro, G., Rossi, E., Janne, P.A., Carnaghi, C., Calandri, C., Bencardino, K., Ligorio, C., Ciardiello, F., Pressiani, T., Destro, A., Roncalli, M., Crino, L., Franklin, W.A., Santoro, A. & Varella-Garcia, M. (2008). EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients. *Ann Oncol*, **19**, 717-23.

Ciardiello, F., Lenz, H.-J., Kohne, C.-H., Heinemann, V., Tejpar, S., Esser, R., Beier, F., Stroh, C., Duecker, K. & Van Cutsem, E. (2014). Effect of KRAS and NRAS mutational status on first-line treatment with FOLFIRI plus cetuximab in patients with metastatic colorectal cancer (mCRC): New results from the CRYSTAL trial. *ASCO Meeting Abstracts*, **32**, LBA443.

Cleries, R., Esteban, L., Borras, J., Marcos-Gragera, R., Freitas, A., Carulla, M., Buxo, M., Puigdefabregas, A., Izquierdo, A., Gispert, R., Galceran, J. & Ribes, J. (2014). Time trends of cancer incidence and mortality in Catalonia during 1993-2007. *Clin Transl Oncol*, **16**, 18-28.

Courtney, K.D., Corcoran, R.B. & Engelman, J.A. (2010). The PI3K pathway as drug target in human cancer. *J Clin Oncol*, **28**, 1075-83.

Cuffy, M., Abir, F. & Longo, W.E. (2006). Management of less common tumors of the colon, Recto, and anus. *Clin Colorectal Cancer*, **5**, 327-37.

Cunningham, D., Humblet, Y., Siena, S., Khayat, D., Bleiberg, H., Santoro, A., Bets, D., Mueser, M., Harstrick, A., Verslype, C., Chau, I. & Van Cutsem, E. (2004). Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*, **351**, 337-45.

Chung, K.Y., Shia, J., Kemeny, N.E., Shah, M., Schwartz, G.K., Tse, A., Hamilton, A., Pan, D., Schrag, D., Schwartz, L., Klimstra, D.S., Fridman, D., Kelsen, D.P. & Saltz, L.B. (2005). Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol*, **23**, 1803-10.

Davies, G., Jiang, W.G. & Mason, M.D. (2001). Matrilysin mediates extracellular cleavage of E-cadherin from prostate cancer cells: a key mechanism in hepatocyte growth factor/scatter factor-induced cell-cell dissociation and in vitro invasion. *Clin Cancer Res*, **7**, 3289-97.

de Lau, W., Barker, N., Low, T.Y., Koo, B.K., Li, V.S., Teunissen, H., Kujala, P., Haegebarth, A., Peters, P.J., van de Wetering, M., Stange, D.E., van Es, J.E., Guardavaccaro, D., Schasfoort, R.B., Mohri, Y., Nishimori, K., Mohammed, S., Heck, A.J. & Clevers, H. (2011). Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*, **476**, 293-7.

De Roock, W., Claes, B., Bernasconi, D., De Schutter, J., Biesmans, B., Fountzilas, G., Kalogeras, K.T., Kotoula, V., Papamichael, D., Laurent-Puig, P., Penault-Llorca, F., Rougier, P., Vincenzi, B., Santini, D., Tonini, G., Cappuzzo, F., Frattini, M., Molinari, F., Saletti, P., De Dosso, S., Martini, M., Bardelli, A., Siena, S., Sartore-Bianchi, A., Tabernero, J., Macarulla, T., Di Fiore, F., Gangloff, A.O., Ciardiello, F., Pfeiffer, P., Qvortrup, C., Hansen, T.P., Van Cutsem, E., Piessevaux, H., Lambrechts, D., Delorenzi, M. & Tejpar, S. (2010). Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*, **11**, 753-62.

Diaz, L.A., Jr., Williams, R.T., Wu, J., Kinde, I., Hecht, J.R., Berlin, J., Allen, B., Bozic, I., Reiter, J.G., Nowak, M.A., Kinzler, K.W., Oliner, K.S. & Vogelstein, B. (2012). The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*, **486**, 537-40.

Douillard, J.Y., Siena, S., Cassidy, J., Tabernero, J., Burkes, R., Barugel, M., Humblet, Y., Bodoky, G., Cunningham, D., Jassem, J., Rivera, F., Kocakova, I., Ruff, P., Blasinska-Morawiec, M., Smakal, M., Canon, J.L., Rother, M., Oliner, K.S., Wolf, M. & Gansert, J. (2010). Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol*, **28**, 4697-705.

Fayard, E., Tintignac, L.A., Baudry, A. & Hemmings, B.A. (2005). Protein kinase B/Akt at a glance. *J Cell Sci*, **118**, 5675-8.

Ferguson, K.M. (2008). Structure-based view of epidermal growth factor receptor regulation. *Annu Rev Biophys*, **37**, 353-73.

Fukuda, A., Wang, S.C., Morris, J.P.t., Folias, A.E., Liou, A., Kim, G.E., Akira, S., Boucher, K.M., Firpo, M.A., Mulvihill, S.J. & Hebrok, M. (2011). Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*, **19**, 441-55.

Gallego, R., Codony-Servat, J., Garcia-Albeniz, X., Carcereny, E., Longaron, R., OHígadoas, A., Tosca, M., Auge, J.M., Gascon, P. & Maurel, J. (2009). Serum IGF-I, IGFBP-3, and matrix metalloproteinase-7 levels and acquired chemo-resistance in advanced colorectal cancer. *Endocr Relat Cancer*, **16**, 311-7.

Glimelius, B., Tiret, E., Cervantes, A. & Arnold, D. (2013). Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, **24 Suppl 6**, vi81-8.

Goldstein, N.I., Prewett, M., Zuklys, K., Rockwell, P. & Mendelsohn, J. (1995). Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res*, **1**, 1311-8.

Gu, G.L., Zhu, X.Q., Wei, X.M., Ren, L., Li, D.C. & Wang, S.L. (2014). Epithelial-mesenchymal transition in colorectal cancer tissue of patients with Lynch syndrome. *World J Gastroenterol*, **20**, 250-7.

He, W., Tan, R.J., Li, Y., Wang, D., Nie, J., Hou, F.F. & Liu, Y. (2012). Matrix metalloproteinase-7 as a surrogate marker predicts renal Wnt/beta-catenin activity in CKD. *J Am Soc Nephrol*, **23**, 294-304.

Heestand, G.M., Murphy, J.D., Moughan, J., Regine, W., Luo, J., Graber, M.S., Kunz, P.L., Fisher, G.A., Guha, C., Lin, B., Mowat, R.B., Gaur, R., Buyyounouski, M.K., Chen, Y., Chang, D.T. & Koong, A. (2014). A novel biomarker panel examining response to adjuvant pancreatic cancer therapy in RTOG 9704. *ASCO Meeting Abstracts*, **32**, 176.

Ii, M., Yamamoto, H., Adachi, Y., Maruyama, Y. & Shinomura, Y. (2006). Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Exp Biol Med (Maywood)*, **231**, 20-7.

Ishikawa, T., Ichikawa, Y., Mitsuhashi, M., Momiyama, N., Chishima, T., Tanaka, K., Yamaoka, H., Miyazakic, K., Nagashima, Y., Akitaya, T. & Shimada, H. (1996). Matrilysin is associated with progression of colorectal tumor. *Cancer Lett*, **107**, 5-10.

Ito, T.K., Ishii, G., Saito, S., Yano, K., Hoshino, A., Suzuki, T. & Ochiai, A. (2009). Degradation of soluble VEGF receptor-1 by MMP-7 allows VEGF access to endothelial cells. *Blood*, **113**, 2363-9.

Jonker, D.J., O'Callaghan, C.J., Karapetis, C.S., Zalcberg, J.R., Tu, D., Au, H.J., Berry, S.R., Krahn, M., Price, T., Simes, R.J., Tebbutt, N.C., van Hazel, G., Wierzbicki, R., Langer, C. & Moore, M.J. (2007). Cetuximab for the treatment of colorectal cancer. *N Engl J Med*, **357**, 2040-8.

Kang, H., O'Connell, J.B., Leonardi, M.J., Maggard, M.A., McGory, M.L. & Ko, C.Y. (2007). Rare tumors of the colon and Recto: a national review. *Int J Colorectal Dis*, **22**, 183-9.

Kantoff, P.W., Higano, C.S., Shore, N.D., Berger, E.R., Small, E.J., Penson, D.F., Redfern, C.H., Ferrari, A.C., Dreicer, R., Sims, R.B., Xu, Y., Frohlich, M.W. & Schellhammer, P.F. (2010). Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*, **363**, 411-22.

Karapetis, C.S., Khambata-Ford, S., Jonker, D.J., O'Callaghan, C.J., Tu, D., Tebbutt, N.C., Simes, R.J., Chalchal, H., Shapiro, J.D., Robitaille, S., Price, T.J., Shepherd, L., Au, H.J., Langer, C., Moore, M.J. & Zalcberg, J.R. (2008). K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*, **359**, 1757-65.

Kolligs, F.T., Bommer, G. & Goke, B. (2002). Wnt/beta-catenin/tcf signaling: a critical pathway in gastrointestinal tumorigenesis. *Digestion*, **66**, 131-44.

Labianca, R., Nordlinger, B., Beretta, G.D., Mosconi, S., Mandala, M., Cervantes, A. & Arnold, D. (2013). Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, **24 Suppl 6**, vi64-72.

Li, X., Qu, L., Zhong, Y., Zhao, Y., Chen, H. & Daru, L. (2013). Association between promoters polymorphisms of matrix metalloproteinases and risk of digestive cancers: a meta-analysis. *J Cancer Res Clin Oncol*, **139**, 1433-47.

Liu, W., Dong, X., Mai, M., Seelan, R.S., Taniguchi, K., Krishnadath, K.K., Halling, K.C., Cunningham, J.M., Boardman, L.A., Qian, C., Christensen, E., Schmidt, S.S., Roche, P.C., Smith, D.I. & Thibodeau, S.N. (2000). Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling. *Nat Genet*, **26**, 146-7.

Lynch, C.C., Hikosaka, A., Acuff, H.B., Martin, M.D., Kawai, N., Singh, R.K., Vargo-Gogola, T.C., Begtrup, J.L., Peterson, T.E., Fingleton, B., Shirai, T., Matrisian, L.M. & Futakuchi, M. (2005). MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. *Cancer Cell*, **7**, 485-96.

Lynch, C.C., Vargo-Gogola, T., Matrisian, L.M. & Fingleton, B. (2010). Cleavage of E-Cadherin by Matrix Metalloproteinase-7 Promotes Cellular Proliferation in Nontransformed Cell Lines via Activation of RhoA. *J Oncol*, **2010**, 530745.

Malvezzi, M., Bertuccio, P., Levi, F., La Vecchia, C. & Negri, E. (2013). European cancer mortality predictions for the year 2013. *Ann Oncol*, **24**, 792-800.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V., Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L., Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz, S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna, M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth, G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz, A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P., Begley, R.F. & Rothberg, J.M. (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376-80.

Markowitz, S.D. & Bertagnolli, M.M. (2009). Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med*, **361**, 2449-60.

Masaki, T., Matsuoka, H., Sugiyama, M., Abe, N., Goto, A., Sakamoto, A. & Atomi, Y. (2001). Matrilysin (MMP-7) as a significant determinant of malignant potential of early invasive colorectal carcinomas. *Br J Cancer*, **84**, 1317-21.

Massova, I., Kotra, L.P., Fridman, R. & Mobashery, S. (1998). Matrix metalloproteinases: structures, evolution, and diversification. *Faseb J*, **12**, 1075-95.

Masui, H., Kawamoto, T., Sato, J.D., Wolf, B., Sato, G. & Mendelsohn, J. (1984). Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res*, **44**, 1002-7.

Maurel, J., Garcia-Albeniz, X., Mendez Mendez, C., Martin-Richard, M., Pericay, C., Vera, R., Aparicio, J., Rubini, M., Cuatrecasas, M. & on behalf of the, G.c.g. (2011). PULSE: An open-label, phase II study assessing double positivity (phospho-insulin-growth factor receptor-1 [pIGF-IR] and matrilysin [MMP7]) expression as a predictive marker of resistance in previously untreated metastatic colorectal cancer (mCRC) wild-type KRAS patients (pts) treated with panitumumab plus mFOLFOX6--A GEMCAD study. *ASCO Meeting Abstracts*, **29**, TPS164.

Maurel, J., Nadal, C., Garcia-Albeniz, X., Gallego, R., Carcereny, E., Almendro, V., Marmol, M., Gallardo, E., Maria Auge, J., Longaron, R., Martinez-Fernandez, A., Molina, R., Castells, A. & Gascon, P. (2007). Serum matrix metalloproteinase 7 levels identifies poor prognosis advanced colorectal cancer patients. *Int J Cancer*, **121**, 1066-71.

Misale, S., Yaeger, R., Hobor, S., Scala, E., Janakiraman, M., Liska, D., Valtorta, E., Schiavo, R., Buscarino, M., Siravegna, G., Bencardino, K., Cercek, A., Chen, C.T., Veronese, S., Zanon, C., Sartore-Bianchi, A., Gambacorta, M., Gallicchio, M., Vakiani, E., Boscaro, V., Medico, E., Weiser, M., Siena, S., Di Nicolantonio, F., Solit, D. & Bardelli, A. (2012).

Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature*, **486**, 532-6.

Miyamoto, S., Nakamura, M., Yano, K., Ishii, G., Hasebe, T., Endoh, Y., Sangai, T., Maeda, H., Shi-Chuang, Z., Chiba, T. & Ochiai, A. (2007). Matrix metalloproteinase-7 triggers the matricrine action of insulin-like growth factor-II via proteinase activity on insulin-like growth factor binding protein 2 in the extracellular matrix. *Cancer Sci*, **98**, 685-91.

Montagut, C., Dalmases, A., Bellosillo, B., Crespo, M., Pairet, S., Iglesias, M., Salido, M., Gallen, M., Marsters, S., Tsai, S.P., Minoche, A., Seshagiri, S., Serrano, S., Himmelbauer, H., Bellmunt, J., Rovira, A., Settleman, J., Bosch, F. & Albanell, J. (2012). Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med*, **18**, 221-3.

Morelli, M.P., Overman, M.J., Dasari, A., Kazmi, S.M.A., Vilar Sanchez, E., Eng, C., Kee, B.K., Deaton, L., Garrett, C.R., Diehl, F., Angenendt, P. & Kopetz, S. (2013). Heterogeneity of acquired KRAS and EGFR mutations in colorectal cancer patients treated with anti-EGFR monoclonal antibodies. *ASCO Meeting Abstracts*, **31**, 3512.

Mori, M., Barnard, G.F., Mimori, K., Ueo, H., Akiyoshi, T. & Sugimachi, K. (1995). Overexpression of matrix metalloproteinase-7 mRNA in human colon carcinomas. *Cancer*, **75**, 1516-9.

Normanno, N., De Luca, A., Salomon, D.S. & Ciardiello, F. (1998). Epidermal growth factor-related peptides as targets for experimental therapy of human colon carcinoma. *Cancer Detect Prev*, **22**, 62-7.

Pinto, D. & Clevers, H. (2005). Wnt, stem cells and cancer in the intestine. *Biol Cell*, **97**, 185-96.

Pinto, D., Gregorieff, A., Begthel, H. & Clevers, H. (2003). Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev*, **17**, 1709-13.

Pryczynicz, A., Gryko, M., Niewiarowska, K., Dymicka-Piekarska, V., Ustymowicz, M., Hawryluk, M., Cepowicz, D., Borsuk, A., Kemona, A., Famulski, W. & Guzinska-Ustymowicz, K. (2013). Immunohistochemical expression of MMP-7 protein and its serum level in colorectal cancer. *Folia Histochem Cytobiol*, **51**, 206-12.

Reya, T. & Clevers, H. (2005). Wnt signalling in stem cells and cancer. *Nature*, **434**, 843-50.

Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M. & Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*, **469**, 415-8.

Schepers, A.G., Snippert, H.J., Stange, D.E., van den Born, M., van Es, J.H., van de Wetering, M. & Clevers, H. (2012). Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science*, **337**, 730-5.

Sidhu, R., Rong, A. & Dahlberg, S. (2013). Evaluation of progression-free survival as a surrogate endpoint for survival in chemotherapy and targeted agent metastatic colorectal cancer trials. *Clin Cancer Res*, **19**, 969-76.

Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J. & Norton, L. (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*, **344**, 783-92.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D. & Clevers, H. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell*, **143**, 134-44.

Sabin, L., MK, G. & C, W. (2009). *TNM Classification of Malignant Tumours. 7th Edition*. Wiley-Blackbell: Oxford.

Sobrero, A.F., Maurel, J., Fehrenbacher, L., Scheithauer, W., Abubakr, Y.A., Lutz, M.P., Vega-Villegas, M.E., Eng, C., Steinhauer, E.U., Prausova, J., Lenz, H.J., Borg, C., Middleton, G., Kroning, H., Luppi, G., Kisker, O., Zubel, A., Langer, C., Kopit, J. & Burris, H.A., 3rd. (2008). EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol*, **26**, 2311-9.

Sparano, J.A., Bernardo, P., Stephenson, P., Gradishar, W.J., Ingle, J.N., Zucker, S. & Davidson, N.E. (2004). Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196. *J Clin Oncol*, **22**, 4683-90.

Szarvas, T., Becker, M., vom Dorp, F., Gethmann, C., Totsch, M., Bankfalvi, A., Schmid, K.W., Romics, I., Rubben, H. & Ergun, S. (2010). Matrix metalloproteinase-7 as a marker of metastasis and predictor of poor survival in bladder cancer. *Cancer Sci*, **101**, 1300-8.

Szarvas, T., Becker, M., Vom Dorp, F., Meschede, J., Scherag, A., Bankfalvi, A., Reis, H., Schmid, K.W., Romics, I., Rubben, H. & Ergun, S. (2011). Elevated serum matrix metalloproteinase 7 levels predict poor prognosis after radical prostatectomy. *Int J Cancer*, **128**, 1486-92.

- Tallant, C., Marrero, A. & Gomis-Ruth, F.X. (2010). Matrix metalloproteinases: fold and function of their catalytic domains. *Biochim Biophys Acta*, **1803**, 20-8.
- Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J.P. & He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. *Nature*, **407**, 530-5.
- Tejpar, S., Lenz, H.-J., Kohne, C.-H., Heinemann, V., Ciardiello, F., Esser, R., Beier, F., Stroh, C., Duecker, K. & Bokemeyer, C. (2014). Effect of KRAS and NRAS mutations on treatment outcomes in patients with metastatic colorectal cancer (mCRC) treated first-line with cetuximab plus FOLFOX4: New results from the OPUS study. *ASCO Meeting Abstracts*, **32**, LBA444.
- Tidyman, W.E. & Rauen, K.A. (2009). The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev*, **19**, 230-6.
- Van Cutsem, E., Kohne, C.H., Hitre, E., Zaluski, J., Chang Chien, C.R., Makhson, A., D'Haens, G., Pinter, T., Lim, R., Bodoky, G., Roh, J.K., Folprecht, G., Ruff, P., Stroh, C., Tejpar, S., Schlichting, M., Nippgen, J. & Rougier, P. (2009). Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*, **360**, 1408-17.

Van Cutsem, E., Nordlinger, B. & Cervantes, A. (2013). Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann Oncol*, **21 Suppl 5**, v93-7.

Wilson, C.L., Heppner, K.J., Rudolph, L.A. & Matrisian, L.M. (1995). The metalloproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. *Mol Biol Cell*, **6**, 851-69.

Yang, X.D., Jia, X.C., Corvalan, J.R., Wang, P. & Davis, C.G. (2001). Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. *Crit Rev Oncol Hematol*, **38**, 17-23.

Yin, Y., Grabowska, A.M., Clarke, P.A., Whelband, E., Robinson, K., Argent, R.H., Tobias, A., Kumari, R., Atherton, J.C. & Watson, S.A. (2010). Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7. *Gut*, **59**, 1037-45.

Zucker, S. & Vacirca, J. (2004). Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev*, **23**, 101-17.



## **II. APÉNDICE**



## **VIII.I LISTADO DE ABREVIATURAS**

- CCR Cáncer colorrectal  
CEA Antígeno Carcinoembrionario  
CR Respuesta completa  
EE Enfermedad Estable  
FISH Hibridación fluorescente in situ  
FT Factor de transcripción  
Gy Gray  
HR Hazard Ratio  
IC Intervalo de Confianza  
MMP Matriz-Metaloproteinasa  
PE Progresión de la enfermedad  
PIP2 Fosfatidil inositol bifosfato  
PIP3 Fosfatidil inositol trifosfato  
PR Respuesta Parcial  
RP Respuesta Parcial  
rpm revoluciones por minuto  
SG Supervivencia Global  
SLE Supervivencia Libre de Enfermedad  
TC Tomografía computarizada  
UICC Unión Internacional Contra el Cáncer

## **VIII.II. LISTADO DE FIGURAS**

Figura 1 : Anatomía del intestino grueso	Página 1
Figura 2 : Corte histológico del intestino grueso	Página 2
Figura 3 : Tipos celulares del epitelio del intestino grueso	Página 3
Figura 4 : Activación de la vía Wnt	Página 6
Figura 5 : Activación de $\beta$ -catenina	Página 7
Figura 6 : Esquema de la estructura de EGFR	Página 9
Figura 7 : Activación de EGFR y reclutamiento de proteínas adaptadoras	Página 10
Figura 8 : Activación de Ras a través de la familia SOS	Página 12
Figura 9 : Vía de RAS-MAPK	Página 13
Figura 10 : Activación de PI3K	Página 16
Figura 11 : Activación de mTORC1	Página 18
Figura 12 : Visión general de las vías activadas por EGFR	Página 19
Figura 13 : Evolución del epitelio intestinal hasta la degeneración maligna	Página 23
Figura 14 : Genes mutados en el cáncer colorrectal	Página 24
Figura 15 : Acción de los anticuerpos contra EGFR	Página 28
Figura 16 : Representación de las MMP	Página 34
Figura 17 : Representación de la técnica ELISA	Página 57
Figura 18 : Determinación de MMP-7. Representación Kaplan-Meier de la Supervivencia libre de Enfermedad	Página 77
Figura 19 : Determinación de MMP-7: Representación Kaplan-Meier de Supervivencia global	Página 78

Figura 20: Determinación de MMP-7: Predicción de la Supervivencia Libre de Enfermedad	Página 81
Figura 21: Mutaciones K467T y R451C en EGFR	Página 89
Figura 22: Amplificación EGFR evaluada por FISH	Página 91
Figura 23: Evolución eventos moleculares paciente 27	Página 94
Figura 24: Evolución eventos moleculares paciente 31	Página 95
Figura 25: Evolución eventos moleculares paciente 8	Página 97

### **VIII.III LISTADO DE TABLAS**

Tabla 1: Clasificación TNM del cáncer colorrectal	Página 25
Tabla 2: Clasificación por estadios del cáncer colorrectal	Página 26
Tabla 3: Características pacientes estudio MMP-7	Página 72
Tabla 4: Determinación de MMP-7. Análisis uni y multivariante de Supervivencia Libre de Enfermedad	Página 75
Tabla 5: Determinación de MMP-7. Análisis uni y multivariante de Supervivencia Global	Página 76
Tabla 6: Supervivencia Libre de Enfermedad y Supervivencia Global según MMP-7	Página 79
Tabla 7: Predicción de Supervivencia Libre de Enfermedad según valores de MMP-7 y afectación ganglionar	Página 82
Tabla 8: Características pacientes estudio cetuximab	Página 84-85
Tabla 9: Estudio molecular de las mutaciones presentes antes y después del tratamiento con cetuximab	Página 86-87
Tabla 10: Resumen de mutaciones y amplificaciones detectadas en las biopsias en relación al tratamiento con cetuximab	Página 87
Tabla 11: Resumen de la frecuencia de las mutaciones detectadas tras el tratamiento con cetuximab	Página 90

## **VIII.IV PUBLICACIONES**

Los resultados de esta tesis doctoral han generado las siguientes comunicaciones científicas:

- 2007 ASCO Annual Meeting. *Serum matrilysin (MMP7) levels are associated with progression, in curatively resected colorectal cancer (CRC) patients.* Abstract 4124.
- 2009 Cancer Gastrointestinal Symposium ASCO. *Matrilysin-based score for predicting recurrence in curatively resected colorectal cancer (CRC).* Abstract 300
- 2014 ASCO Annual Meeting. *Evolution of heterogeneous mechanisms of acquired resistance to cetuximab-based therapy in colorectal cancer.* Abstract 3526.

Asimismo, parte de los resultados de este estudio han sido publicados en:

Martínez-Fernandez A, García-Albeniz X, Pineda E, Visa L, Gallego R, Codony-Servat J, Augé JM, Longarón R, Gascón P, Lacy A, Castells A, Maurel J. (2009). Serum matrilysin levels predict outcome in curatively resected colorectal cancer patients. *Ann Surg Oncol*, **16**, 1412-20.

El doctorando además ha generado las siguientes publicaciones durante el periodo de tesis doctoral (2012-actualidad), pero no relacionadas con el proyecto de tesis aquí presentada:

Martínez-Fernández A. The long road to bladder sparing in muscle invasive cancer.

(2013) *Lancet Oncol* **14**, 795-6

Rodig SJ, Cheng J, Wardzala J, DoRosario A, Scanlon JJ, Laga AC, Martinez-Fernandez A, et al. Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus (2012), *J Clin Invest* **122**, 4645-53.