Introduction

Cardiotrophin-1 (CT-1/CTF1) is a member of the interleukin (IL)-6 family of cytokines that stimulates STAT-3 phosphorylation in cells bearing the cognate receptor. We report that Ctf1−/− mice (hereby referred to as CT-1−/− mice) are resistant to the hepatic engraftment of MC38 colon carcinoma cells, while these cells engraft normally in the mouse subcutaneous tissue. Tumor intake in the liver could be enhanced by the systemic delivery of a recombinant adenovirus encoding CT-1, which also partly rescued the resistance of CT-1−/− mice to the hepatic engraftment of MC38 cells. Moreover, systemic treatment of wild-type (WT) mice with a novel antibody-neutralizing mouse cT-1 also reduced engraftment of this model. Conversely, experiments with Panc02 pancreatic cancer and B16-OVA melanoma cells in CT-1−/− mice revealed rates of hepatic engraftment similar to those observed in WT mice. The mechanism whereby CT-1 renders the liver permissive for MC38 metastasis involves T lymphocytes and natural killer (NK) cells, as shown by selective depletion experiments and in genetically deficient mice. However, no obvious changes in the number or cell killing capacity of liver lymphocytes in CT-1−/− animals could be substantiated. These findings demonstrate that the seed and soil concept to understand metastasis can be locally influenced by cytokines as well as by the cellular immune system.

Cardiotrophin-1 (CT-1/CTF1) is a member of the interleukin-6 (IL-6) family of cytokines that stimulates STAT-3 phosphorylation in cells bearing the cognate receptor. We report that Ctf1−/− mice (hereby referred to as CT-1−/− mice) are resistant to the hepatic engraftment of MC38 colon carcinoma cells, while these cells engraft normally in the mouse subcutaneous tissue. Tumor intake in the liver could be enhanced by the systemic delivery of a recombinant adenovirus encoding CT-1, which also partly rescued the resistance of CT-1−/− mice to the hepatic engraftment of MC38 cells. Moreover, systemic treatment of wild-type (WT) mice with a novel antibody-neutralizing mouse cT-1 also reduced engraftment of this model. Conversely, experiments with Panc02 pancreatic cancer and B16-OVA melanoma cells in CT-1−/− mice revealed rates of hepatic engraftment similar to those observed in WT mice. The mechanism whereby CT-1 renders the liver permissive for MC38 metastasis involves T lymphocytes and natural killer (NK) cells, as shown by selective depletion experiments and in genetically deficient mice. However, no obvious changes in the number or cell killing capacity of liver lymphocytes in CT-1−/− animals could be substantiated. These findings demonstrate that the seed and soil concept to understand metastasis can be locally influenced by cytokines as well as by the cellular immune system.
characterize the role of the gp130 signaling cytokines during inflammation, suggesting that this receptor constitutes a prominent factor in balancing pro- and anti-inflammatory responses. Moreover, while there are studies suggesting that gp130-mediated signaling may be critical in oncogenesis, other reports demonstrate the ability of some gp130 family cytokines, such as OSM and IL-27, to block tumor progression.² It is important to emphasize that gp130-mediated signaling exerts a broad range of biological effects in cancer. The gp130-mediated activation of STAT3 by a cognate cytokine or upon somatic mutation of the receptor-encoding gene is oncogenic and contributes to disease progression in several models of cancer. Conversely, the overexpression of IL-27 has been shown to result in an increased clearance of multiple tumor type in mice, a phenomenon that was associated with enhanced antitumor CD8⁺ T-cell and natural killer (NK)-cell responses.¹⁰

In this study, we found that the adenovirus-mediated delivery of CT-1 promotes the growth of MC38 colon carcinoma cells in the mouse liver, while the neutralization of CT-1 with a monoclonal antibody mitigates engraftment. Altogether, these results suggest that CT-1 may constitute a valuable therapeutic target. Moreover, CT-1-null mice rejected the engraftment of MC38 colon carcinoma cells, either as injected in the hepatic parenchyma or disseminating upon intrasplenic injection. This resistance was not observed in the subcutaneous tissue and was found to be dependent on T and NK cells. These findings open a new field for the study of CT-1, whose mRNA is expressed at baseline levels in the healthy liver.⁵

Results

Lack of CT-1 results in the rejection of MC38 colon carcinomas engrafted in the mouse liver. MC38 cells are a colon carcinoma cell line that readily grows in the liver of C57BL/6 mice, both when injected directly into the hepatic parenchyma and when injected in the spleen to metastasize through the portal vein. To test whether endogenous CT-1 could play a role in the engraftment of MC38 colon carcinoma cells, the liver of CT-1-null (Ctf1⁻/⁻, hereafter referred to as CT-1⁻/⁻) and wild-type (WT) mice was inoculated with tumor cells. As shown in Figure 1A, five out of seven CT-1⁻/⁻ mice backcrossed into C57BL/6 background for ten generations rejected tumor cells, while in WT C57BL/6 and Il6⁻/⁻ mice, engrafted cells gave rise to lethal liver tumors in three-four weeks. In order to completely exclude alloreactive rejection mechanisms, animals were further backcrossed to C57BL/6 for two additional generations selecting for the most C57BL/6 background-matched breeders by analyses of microsatellite polymorphism. Similar results were observed again with six out of eight CT-1⁻/⁻ mice, completely rejecting tumors that readily grew in littermate controls hosted in the same cages (Fig. 1B). CT-SCAN images performed on day 14 after tumor-cell inoculation show an example of each type of mice, with a 3D computer-assisted reconstruction indicating the presence of early progressing liver metastases (Fig. 1C). Interestingly, WT, CT-1⁻/⁻ and Il6⁻/⁻ mice challenged with the same, lethal dose of MC38 cells in the subcutaneous tissue all developed rapidly progressing lethal tumors.

The resistance to the hepatic engraftment of tumor cells exhibited by CT-1⁻/⁻ mice was related to the MC38 cell line, as we could not observe a similar phenotype when B16-OVA (melanoma) and Panc02 (pancreatic carcinoma) cells, representing to tumor types that are naturally prone to hepatic metastasis, were used (Fig. S1).

Interestingly, when CT-1⁻/⁻ mice that had rejected an intraperitoneal injection of MC38 cells were rechallenged in the dermis, eight out of ten mice did not develop tumors, while these cells readily engrafted in the subcutaneous tissue of CT-1⁻/⁻ tumor-naïve animals as well as in WT mice (Fig. 2). These findings suggest the development of some type of immunological memory or vaccine-like effect following the first exposure of CT-1⁻/⁻ livers to tumor cells.

The adenoviral delivery of CT-1 promotes hepatic engraftment of MC38 cell-derived tumors. To further explore the role of CT-1 in the progression of metastasis, we used a liver-targeting gene transfer approach based on a first-generation recombinant adenovirus encoding CT-1 (AdCT-1).³ AdCT-1 was administered 48h before the hepatic inoculation of MC38 tumor cells. Our data show that AdCT-1 significantly enhances the engraftment and growth of tumor cells at day 14 following inoculation as compared with a LacZ-coding adenovirus AdLacZ = used as a control condition. Photographs of the abdomen of WT euthanized mice (Fig. 3A) show two examples per condition of hepatic tumor engraftment. Moreover, AdCT-1 given 2 d before the inoculation of tumor cells was able to partly rescue the resistance to engraftment of CT-1⁻/⁻ mice (Fig. 3B). Hence, CT-1 gene transfer gave rise to progressing MC38 tumors in the liver of CT-1⁻/⁻ mice.

At this point, we wondered whether CT-1 would constitute a necessary trophic factor for the hepatic growth of MC38 tumor cells. However, experiments performed in vitro showed that MC38 cells proliferate equally in the presence and in the absence of CT-1 (Fig. S2A). Of note, neutralizing CT-1 with a specific monoclonal antibody did not change the proliferation rate of cultured MC38 tumor cells (Fig. S2B).

Anti-CT-1 antibodies mitigate the progression of MC38 cell-derived liver metastasis. In order to explore the potential of CT-1 as a therapeutic target, we generated CT-1-specific monoclonal antibodies in CT-1⁻/⁻ null mice. The MAB19 antibody was selected as it efficiently bound to both mouse and rat CT-1 in ELISA assays (Fig. 4A). MAB19 was neutralizing, since it partly inhibited the phosphorylation of STAT3 as induced by a short pulse (30 min) of CT-1 in mouse hepatocarcinoma Hepa 1–6 cells that had previously depleted from serum and growth factors overnight. Thus, MAB19 can neutralize the direct effects of recombinant CT-1, albeit not completely in these assays, as revealed by immunoblotting for the phosphorylated form of STAT3 (P-STAT3) (Fig. 4B).

Next, we intraperitoneally administered MAB19 to mice on days -3, -1, 1, 3 and 6 (day = hepatic injection of MC38 cells). As can be seen in Figure 5A, WT mice receiving MAB19 but not animals treated with a control antibody manifested a delay in the growth of liver MC38 cell-derived metastases, as assessed by surgical examination performed on day 14. Such an effect was
tumor nodules were observed in CT-1−/− animals than in their WT counterparts. Figure 6A shows a histological image of representative tumor nodules, while Figure 6B shows quantitative analyses of tumor area in multiple non-serial liver slides stained with hematoxylin and eosin, which were consistent with the inspection of formalin-fixed organs. These data confirm that CT-1−/− livers are less prone to accept MC38 cell-derived metastatic emboli incoming from the spleen via the portal circulation than WT organs.

Involvement of the immune system in the pro-engraftment effects of CT-1. In order to study the potential involvement of
similar results in CT-1−/− and WT mice (Fig. S3). Moreover, mononuclear leukocytes purified from the liver of CT-1−/− and WT mice, did not differ in their ability to kill MC38 and YAC-1 target cells, neither in baseline conditions nor when their cytolytic activity was boosted by the treatment of mice with polyI:C, indicating no obvious alterations in the cytolytic functions of intrahepatic CT-1−/− vs. WT immune cells (Fig. S4).

A possible clue to clarify these observations may come from the differential response of CT-1−/− mice to the hepatic engraftment of MC38, Panc02 and B16-OVA cells. Indeed, MHC Class I (H-2Kb and Db) was robustly expressed on cultured MC38 cells, but not on Panc02 (weak expression) and B16-OVA (no expression) cells. However it could be induced by IFN-γ in every case (Fig. S5). One additional element in this setting could be represented by the ability of tumor cells to produce CT-1 themselves. To clarify this aspect, culture supernatants from the above-mentioned cell lines were assayed for CT-1 content by ELISA. We found that all three cell lines secrete CT-1, although MC38 does so to much higher values, particularly when cultured in the presence of fetal bovine serum (Fig. S6A). The amount of secreted CT-1 correlates was found to correlate with CT-1 mRNA expression levels as assessed by quantitative RT-PCR (Fig. S6B).

In the immune system in the observed phenotype, we selectively depleted (by means of specific monoclonal antibodies) T-cell subsets and NK cells. Figure 7A shows experiments performed by inoculating MC38 cells in the spleen and using the surface fraction of the liver covered with tumors, on day 14, as a read-out. In line with previous observations, the area of CT-1−/− livers covered with tumor cells was lower than that of WT organs. Interestingly, the depletion of CD4+ plus CD8+ cells as well as of NK cells (with an anti-asialo GM1 antibody), resulted in a massive tumor progression in most instances. Indeed, the dual depletion of NK and T cells exerted the most dramatic effect, with some livers that were completely covered by tumor lesions at sacrifice. Next, these experiments were performed following the direct intrahepatic inoculation of tumor cells and using the size of the tumor nodules on day 14 as a readout. Results shown in Figure 7B render are comparable to those obtained with the portal vein dissemination model and indicate that T cells and NK cells (in this case depleted with an anti-NK1.1 monoclonal antibody) are involved in the resistance of CT-1−/− mice to the hepatic engraftment of MC38 tumor cells.

This being said, the intrahepatic percentage and absolute number of CD4+ and CD8+ T cells as well as of NK cells were similar results in CT-1−/− and WT mice (Fig. S3). Moreover, mononuclear leukocytes purified from the liver of CT-1−/− and WT mice, did not differ in their ability to kill MC38 and YAC-1 target cells, neither in baseline conditions nor when their cytolytic activity was boosted by the treatment of mice with polyI:C, indicating no obvious alterations in the cytolytic functions of intrahepatic CT-1−/− vs. WT immune cells (Fig. S4).

A possible clue to clarify these observations may come from the differential response of CT-1−/− mice to the hepatic engraftment of MC38, Panc02 and B16-OVA cells. Indeed, MHC Class I (H-2Kb and Db) was robustly expressed on cultured MC38 cells, but not on Panc02 (weak expression) and B16-OVA (no expression) cells. However it could be induced by IFN-γ in every case (Fig. S5). One additional element in this setting could be represented by the ability of tumor cells to produce CT-1 themselves. To clarify this aspect, culture supernatants from the above-mentioned cell lines were assayed for CT-1 content by ELISA. We found that all three cell lines secrete CT-1, although MC38 does so to much higher values, particularly when cultured in the presence of fetal bovine serum (Fig. S6A). The amount of secreted CT-1 correlates was found to correlate with CT-1 mRNA expression levels as assessed by quantitative RT-PCR (Fig. S6B).
Although we observed an involvement of lymphocytes, we could not detect gross differences in the number or function of hepatic lymphocytes in WT vs. CT-1−/− mice. Hence, the observed discrepancies may be explained by a mechanism that depend on the local innate response against incoming MC38 tumor cells, followed by a T cell-mediated adaptive response. Irrespective of this hitherto unclear aspect, our data unravel a previously unknown effect of CT-1 in determining the hepatic permissiveness to metastatic colonization by colorectal cancer cells.

Discussion

The metastatic colonization of the liver is a common and often fatal outcome of colorectal cancer.11,12 In the same setting, an abundant infiltration of the tumor mass with antigen-experienced CD8+ T cells as well as elevated levels of chemotactic chemokines correlate with improved prognosis independent of multiple additional factors.13,14 NK cells among the tumor infiltrate are also a favorable finding.15 In this study, we report the surprising observation that CT-1−/− mice are resistant to liver engraftment by MC38 colorectal carcinoma cells. This effect was initially attributed to a putative activity of CT-1 on tumor cells, since CT-1 could—at least theoretically—operate as an anti-apoptotic or trophic factor. However, we observed that the exogenous administration of CT-1 to MC38 cells does not increase proliferation and that CT-1 neutralization with specific monoclonal antibody appears not to affect MC38 cell cultures. Therefore, the effects of CT-1 on liver engraftment turned out to be indirect and to pertain the liver as a soil for the metastatic seed.16

We have previously observed that healthy livers exhibit baseline expression levels of the CT-1-coding mRNA, hence being “ready” to receive metastatic cells. Cirrhotic livers produce less CT-1 than their healthy counterparts (unpublished observations), and it is known that cirrhotic livers are comparatively less prone to undergo metastatic colonization.17,18 Conversely, Okuno and colleagues19 demonstrated that the activation of the innate immune system with recombinant cytokines may prevent hepatic metastasis. Taken together, these findings suggest that the activation of innate immune effectors within the liver might inhibit metastatic colonization. Hence, local factors that repress innate antitumor responses would facilitate the hepatic engraftment of metastases. It should be noted that cytokines and their receptors constitute a very complex signaling system, implying that we may be ignoring key factors to interpret these results. For instance, it has been reported that tumor cells can release soluble forms of the gp130 receptor component that is shared by IL-6 and CT-1. The cytokine and its receptor act in solution as a molecular complex with modified functional properties.20,21 The role of these molecular interplays will need to be addressed in subsequent investigations.

In addition to results obtained with knockout mice, genetic evidence for a critical role of CT-1 in the hepatic engraftment of colorectal carcinoma cells was provided by the transfer of a CT-1 expression cassette encoded by a recombinant adenovirus that infects most hepatocytes when given intravenously.3,4 Gene transfer not only enhanced tumor growth in WT mice, but also rescued part of the resistance to MC38 cell-derived liver metastases of CT-1−/− mice. The recombinant adenovirus was given two days before tumor inoculation in order to precondition the soil to receive incoming tumor cells. Curiously, we could not obtain similar results with other tumors syngenic to C57BL/6 mice and known to generate hepatic metastases. The proportion of tumors that are sensitive to the effects of CT-1 remains to be determined, and at present it cannot be excluded that this may be a selective feature of colon cancer or even a peculiarity of MC38 cells. In this regard, a thorough examination of further C57BL/6-transplantable tumors, including sarcomas and lymphomas, is ongoing.

Another peculiar observation was that the subcutaneous tissue of CT-1−/− mice is perfectly permissive for the engraftment of
MC38 cells. This might be related to differential baseline levels of CT-1 in the dermis and the liver. Additional explanation for this observation may be linked to the high density of immune cells in the liver.22

To study the therapeutic potential of our findings, we have developed a CT-1-neutralizing monoclonal antibody. This was a difficult task, mostly owing to the near identical sequence of CT-1 across species. To circumvent this issue, the antibody MAB19 was generated by immunizing CT-1−/− mice. Alternatively, pro -

Mice. CT-1−/− mice (provided by Dr. Pennica, Genentech) were backcrossed into a C57BL/6 background for 11 generations. The CT-1 genotype was confirmed in the breeders by genomic DNA isolation and polymerase chain reaction as previously described.26 Il2−− mice and C57BL/6 mice were originally obtained from The Jackson Laboratory (Bar Harbor) and bred in our animal facility. When indicated, CT-1−/− breeders were selected for microsatellite matching with C57BL/6 pure backgrounds (Bioonsta). Rag2−− Il2ry−− mice were obtained from The Jackson Laboratory and were bred in our animal facility under pathogen-free conditions. Mice that were obtained from The Jackson Laboratory were used between 6 and 14 weeks of age. Animal experiments were performed in accordance with Spanish laws and approval was obtained from the animal experimentation committee of the University of Navarra (Study 034/10 approval).

Tumor experiments. MC38, B16-OVA and Panc02 carcinoma cells have been previously described.27−29 Cells are grown...
The thermocycling conditions for the targets were as follows: denaturing at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 90 sec. Reactions were run on a QX100 Droplet Digital PCR System (Bio-Rad Laboratories, 186–3001). β-actin was employed to normalize the amount of RNA used in each reaction. The amount of each transcript was expressed as the n-fold difference relative to the control transcript coding for β-actin (2^ΔCt, where ΔCt represents the difference in threshold cycle between the control and target gene).

ELISA. ELISA for the detection of CT-1 was performed in supernatants from cell (MC38, Panc02 and B16-OVA) cultures, as previously described.3

Cytotoxicity assays. Cytotoxic activity against MC38 and YAC-1 cells was measured by the standard 5 h sodium chromate ([51Cr] release assay. One million target cells were labeled with 50 μCi [51Cr] (PerkinElmer, NEZ020005MC) for 1 h at 37°C, washed and resuspended in RPMI 1640 (Gibco Invitrogen) containing 10% FBS from Sigma-Aldrich. Liver mononuclear
leukocytes from WT and CT-1−/− mice were used as effector cells. These cells were resuspended in the same medium and placed at various E:T ratios. Labeled target cells were added to each well at a concentration of 3 × 10^3 cells/well for a total volume of 0.2 mL/well. After 5 h incubation, the release of [51Cr] into the supernatant was quantified with a microplate scintillation counter (Packard TopCount, PerkinElmer, C990201). The percentage of cytotoxicity was calculated as the percentage of [51Cr] release using the following equation: (experimental release – spontaneous release) / (maximum release – spontaneous release) × 100.

**Surgical inoculation and follow up of tumors.** A single cell suspension was prepared in phosphate buffered saline and kept on ice. Mice were anesthetized using inhaled isoflurane (Esteve, 13400264). A small nick was made in the skin and the abdominal wall musculature was grasped and lifted up. The abdominal cavity was accessed and a single blade of the scissors used to push the intra-abdominal contents away. The liver is identified and exposed. A 29 G or fine needle (Hamilton Company, 7637–01) was used to inject a 25 μL volume of cell suspension. Finally, the liver was returned to the abdominal cavity and mouse was sutured. For tumor size studies, mice underwent a second laparotomy or were euthanized for necropsy.
Formalin-fixed, paraffin tissue sections were stained with hematoxylin and eosin (H&E). Quantification of metastasis was performed using a computer-based image analysis system equipped with Metamorph software (Universal Imaging Corp.). Briefly, the images of H&E staining were acquired at $\times 100$ ($\times 10$ ocular and $\times 20$ objective) directly from slides to a computer using a Zeiss (Carl Zeiss, Inc.) microscope.

Proliferation assays. MC38 cells were cultured in 96-well plates in RPMI 1640 medium. Later, [methyl-3H]thymidine uptake was determined by the addition of 1 $\mu$Ci of [methyl-3H] thymidine (25 Ci mmol$^{-1}$; GE Healthcare, TRK300–5MCI) for 16–20 h. At the end of the labeling time, $[^{3}H]$thymidine uptake was determined by transferring cells to 96-well filter microplates (Unifilter-96 GF/C, PerkinElmer, 6005174) and adding 25 $\mu$L of scintillation liquid (Microscint O, PerkinElmer, 6013611) to measure radioactivity.

Isolation of liver leukocytes. Livers from different mice were surgically harvested. Minced liver lobes were incubated in collagenase D (Roche, 1088–866) and DNase I (Roche, 1284–982) for 15 min at 37°C. Dissociated cells were passed through a 70 $\mu$m nylon mesh filter (BD Falcon, BD Bioscience, 352350) and washed. Dead cells, debris and hepatocytes were then removed with Percoll gradients (Sigma-Aldrich, P7828). Cells were also treated with ACK lysing buffer (Lonza, 10–548E) to remove red blood cells and washed before further analysis.

Administration of AdCT-1. AdCT-1 and AdLacZ were generated$^{10}$ and expanded as previously described. $^{3}$ Animals received residual red cells and washed before further analysis.

Generation of MAB19. CT-1−/− mice were immunized with mouse CT-1 (R&D, 438-CT-050/CF) and hybridomas were produced as described$^{31}$ by conventional fusion techniques. Monoclonal antibodies were selected by ELISA and immunoblotting.

Indirect ELISA for screening of monoclonal anti-CT-1. Briefly, high binding microtiter plates (Greiner, M2936–100EA) were coated with CT-1 (1 $\mu$g/mL) in 0.06 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. Plates were washed 3 times with PBS-Tween 0.05% (PBS-T) and blocked with PBS plus 0.5% Casein (PBS-CAS) for 2 h at 37°C. Antibody supernatants were added to the wells in duplicate and incubated for 1 h at 37°C. After washing, plates were incubated with Goat IgG anti-mouse IgG antibody labeled with peroxidase (Pierce), diluted at 1:10,000 in PBS-T, for 1 h at 37°C. After washing again the reaction was revealed by adding 0.01 M 2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS, Roche, A9941) in citrate buffer containing 0.03% H$_2$O$_2$, and optical density (OD) was determined at 405 nm. Quality, positive and negative controls were included in each plate. The cut off was established as the mean absorbance values for irrelevant IgG plus three standard deviations.

Immunoblotting assays. CT-1 was separated on 12% SDS-PAGE under reducing conditions, and electrotransferred to 0.2 $\mu$m nitrocellulose membranes. Non-specific interactions were blocked by 0.5% casein in PBS incubation, for 2 h at room temperature. Nitrocellulose membrane was then incubated with hybridoma supernatants through a Multiscreen device (Bio-Rad, 170–4017). IgG binding was detected by incubating a secondary antibody, Goat anti-mouse IgG labeled with peroxidase (Pierce), diluted at 1:10,000 in PBS-tween, for 1 h at room temperature. Reaction was revealed by adding western Lightning-ECL peroxidas-chemiluminescent substrate (Perkin Elmer, NEL11001EA). Preimmune sera and culture media were added as negative controls. A commercial antibody against CT-1 (R&D) was used as positive control.

Statistics. Comparisons were made with paired Student’s t-tests and log-rank using Prism software (Graph Pad Software). p values are reported.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Elena Ciordia and Eneko Elizalde are acknowledged for excellent animal facility management, as well as technical help by Arantza Azpilikueta. Financial support was from MEC/MICINN (SAF2005–03131 and SAF2008–03294), Departamento de Educación del Gobierno de Navarra, Departamento de Salud del Gobierno de Navarra (Beca Ortiz de Landázuri). Redes temáticas de investigación cooperativa RETIC (RD06/0020/0065), Fondo de investigación sanitaria (FIS PI060932 and PI10/01516), European commission 7th framework program (ENCITE) and SUDOE-IMMUNONET, Fundacion Mutua Madrileña, Programa “Tú eliges: tú decides” de Caja Navarra and “UTE for project FIMA.” MS-H receives a Ramon y Cajal contract from Ministerio de Educación y Ciencia. CA is supported by Fundación Científica de la Asociación Española Contra el Cáncer (AECC).

Supplemental Material
Supplemental material may be found here: www.landesbioscience.com/journals/oncoimmunology/article/22504/
7. Moreno-Aliga MJ, Pérez-Echarri N, Marcos-Gómez B, Larequi E, Gil-Bea FJ, Violler B, et al. Cardiotrophin-1 promotes multidrug resistance in breast cancer cells. Cancer Res 2001; 61:8851-8; PMID:11751408.

8. Conze D, Weiss L, Rogen PS, Bhushan A, Weaver D, Johnson P, et al. Autocrine production of interleukin 6 causes multidrug resistance in breast cancer cells. Cancer Res 2001; 61:8851-8; PMID:11751408.

9. Yang ZF, Lau CK, Ngai P, Lam SP, Ho DW, Poon RT, et al. Cardiotrophin-1 enhances regeneration of cirrhotic liver remnant after hepatectomy through promotion of angiogenesis and cell proliferation. Liver Int 2008; 28:622-31; PMID:18312290; http://dx.doi.org/10.1111/j.1478-3231.2008.01687.x.

10. Matsui M, Kishida T, Nakano H, Yoshimoto K, Shit-Ya M, Shimada T, et al. Interleukin-27 activates natural killer cells and suppresses NK-resistant head and neck squamous cell carcinoma through inducing antibody-dependent cellular cytotoxicity. Cancer Res 2009; 69:2523-30; PMID:19244121; http://dx.doi.org/10.1158/0008-5472.CAN-08-2793.

11. Gangadhar T, Shildky RL; Medsca. Molecular markers to individualize adjuvant therapy for colon cancer. Nat Rev Clin Oncol 2010; 7:318-25; PMID:20404028; http://dx.doi.org/10.1038/nrclinonc.2010.62.

12. Masi G, Fornaro L, Caparello C, Falcone A. Liver metastases from colorectal cancer: how to best complement medical treatment with surgical approaches. Future Oncol 2011; 7:1299-323; PMID:20420420; http://dx.doi.org/10.2217/fon.11.108.

13. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12:298-306; PMID:22419253; http://dx.doi.org/10.1038/nrc3245.

14. Pagès F, Kirillovsky A, Mlecnik B, Audaber M, Tissoloni M, Bindea G, et al. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol 2009; 27:5944-51; PMID:19858404; http://dx.doi.org/10.1200/JCO.2008.19.6147.

15. Camus M, Tissoloni M, Mlecnik B, Pagès F, Kirillovsky A, Berger A, et al. Coordination of intratumoral immune reaction and human colorectal cancer recurrence. Cancer Res 2009; 69:2685-93; PMID:19258510; http://dx.doi.org/10.1158/0008-5472.CAN-08-2654.