

# Immunological effects of chemotherapy in spontaneous breast cancers

Guido Kroemer<sup>1,2,3,4,5,\*</sup>, Lorenzo Galluzzi<sup>1,2,6</sup>, and Laurence Zitvogel<sup>6,7,8,9</sup>

<sup>1</sup>Université Paris Descartes/Paris V; Sorbonne Paris Cité; Paris, France; <sup>2</sup>Equipe 11 labellisée par la Ligue Nationale contre le Cancer; Centre de Recherche des Cordeliers; Paris, France; <sup>3</sup>INSERM, U848; Villejuif, France; <sup>4</sup>Metabolomics and Cell Biology Platforms; Gustave Roussy; Villejuif, France; <sup>5</sup>Pôle de Biologie; Hôpital Européen Georges Pompidou; AP-HP; Paris, France; <sup>6</sup>Gustave Roussy Cancer Campus; Villejuif, France; <sup>7</sup>INSERM, U1015; Villejuif, France; <sup>8</sup>Université Paris-Saclay; Faculté de Médecine; Le Kremlin Bicêtre, France; <sup>9</sup>Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 507; Villejuif, France

**Keywords:** HER2, CD8<sup>+</sup> T lymphocytes, MMTV-*NeuT* mice, oncogene addiction, p53, trastuzumab

In 2012, the research group lead by Karin de Visser at the Netherlands Cancer Institute reported that chemotherapeutic regimens based on cisplatin, oxaliplatin or doxorubicin are equally efficient against breast cancer developing in normal, immunocompetent mice and in mice lacking recombination activating gene 2 (*Rag2*), which codes for a recombinase required for the generation of B and T lymphocytes.<sup>1</sup> In this setting, de Visser and collaborators characterized 2 rather distinct models of breast cancer: (1) the MMTV-*NeuT* model, in which mammary carcinogenesis is driven by the (over)expression of the rat oncogene *Neu* (the ortholog of human *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 2, *ERBB2*, best known as *HER2*) under the control of the mammalian mammary tumor virus (MMTV) promoter, in BALB/c mice; and (2) the *KI4cre; Cdh1<sup>fllox/fllox</sup>; Trp53<sup>fllox/fllox</sup>* model, relying on the epithelial cell-specific, Cre recombinase-dependent knockout of the oncosuppressor genes cadherin 1 (*Cdh1*) and transformation-related protein 53 (*Trp53*, best known as *p53*), in FVB/N mice. In both these experimental paradigms, the presence or absence of *Rag2* (and hence the functionality of B and T lymphocytes) had no impact on the incidence of spontaneous breast carcinomas,<sup>2</sup> and failed to affect the lifespan-extending effects of repeated cycles of chemotherapy.<sup>1</sup> These findings were used to argue against our hypothesis that the efficacy of anticancer therapy

often, if not always, relies on the (re) instatement of immunosurveillance.<sup>3-11</sup> In essence, the results published by de Visser and colleagues seemingly corroborated the idea that conventional chemotherapeutics mediate optimal antineoplastic effects in the absence of any sizeable contribution from the immune system. One of the recurrent arguments used by the de Visser's team in support of their findings was to say that transplantable tumor models, which we have been using for most of our studies, would be inappropriate to reflect the human system, and that de novo carcinoma models (such as those that they extensively employed) would be more relevant from a clinical perspective.

Where do we stand now, at the beginning of 2014, with regard to anticancer immune responses induced by chemotherapy against breast cancers spontaneously developing in mice and humans? First of all, a careful examination of the literature on the MMTV-*NeuT* model supports the notion that immunosurveillance controls breast carcinogenesis and contributes to the efficacy of anticancer chemotherapy in this setting. As early as in 2005, George Prendergast and colleagues (from the The Lankenau Institute for Medical Research) demonstrated that the combination of paclitaxel and 1-methyl-*DL*-tryptophan (an inhibitor of the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1) exerts synergistic anticancer effects that are lost upon the depletion of CD4<sup>+</sup> T cells with a specific monoclonal antibody.<sup>12</sup> In line with this notion, the

concomitant depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells appears to accelerate oncogenesis in MMTV-*NeuT* mice, while the blockade of interleukin-13, an immunosuppressive cytokine, retards the oncogenic process in this model.<sup>13</sup> It is difficult to understand why the pharmacological depletion of T lymphocytes (achieved by the injection of antibodies specific for CD4 and CD8) would promote *Neu*-driven oncogenesis,<sup>13</sup> while the lack of mature T and B cells (resulting from the absence of *Rag2*) would fail to do so.<sup>1</sup> One possibility is that the absence of B lymphocytes, which accelerate oncogenesis in some models,<sup>14</sup> might have neutralized the impact of T-cell depletion. Alternatively, animal facility-dependent differences in the murine microbiome (and hence in the general tone of the immune system) may have affected the efficacy of anticancer immunosurveillance. In another model of transgene-driven breast cancer (MMTV-*rtTA*; TetO-*PyMT*; IRES-*Luc*), which relies on the tetracycline-inducible expression of the polyoma middle T (*PyMT*) oncogene, the depletion of regulatory T cells results in a significant inhibition of tumor growth and metastatic dissemination,<sup>15</sup> supporting the relevance of immunosurveillance in mammary carcinogenesis.

For the present discussion, however, it is more important to note that Wolfgang Doppler and collaborators (from the University of Innsbruck) have recently reported that the treatment of MMTV-*NeuT* mice with doxorubicin or lapatinib

\*Correspondence to: Guido Kroemer; kroemer@orange.fr

Submitted: 12/01/2013; Accepted: 12/01/2013; Published Online: 12/31/2013

Citation: Kroemer G, Galluzzi L, Zitvogel L. Immunological effects of chemotherapy in spontaneous breast cancers. *Oncoimmunology* 2013; 2:e27158; <http://dx.doi.org/10.4161/onci.27158>

(a non-specific inhibitor of HER2 currently approved for use in patients with HER2<sup>+</sup> breast carcinoma), alone or in combination, mediates antitumor effects that disappear upon the depletion of CD8<sup>+</sup> (but not CD4<sup>+</sup>) T lymphocytes.<sup>16</sup> At a first glance, these results are at odds with those reported by the group of Karin de Visser.<sup>1</sup> However, it remains possible that this discrepancy originates from the fact that Doppler and colleagues treated tumor-bearing MMTV-*NeuT* mice with one dose of doxorubicin,<sup>16</sup> while de Visser et al. employed at least three cycles of chemotherapy,<sup>1</sup> which might have induced a severe state of immunosuppression. Interestingly, the knockout of signal transducer and activator of transcription 1 (*Stat1*) abolished not only the therapeutic effects of doxorubicin and lapatinib against *Neu*-driven breast carcinomas, but also the chemotherapy-induced infiltration of neoplastic lesions by T lymphocytes.<sup>16</sup> Thus, the pharmacological inhibition of *Neu* by lapatinib in MMTV-*NeuT* mice mediated therapeutic effects that relied upon the immune system, confirming previous results obtained with HER2-targeting antibodies.<sup>17,18</sup>

## References

- Ciampricotti M, Hau CS, Doornebal CW, Jonkers J, de Visser KE. Chemotherapy response of spontaneous mammary tumors is independent of the adaptive immune system. *Nat Med* 2012; 18:344-6, author reply 346; PMID:22395693; <http://dx.doi.org/10.1038/nm.2652>
- Ciampricotti M, Vrijland K, Hau CS, Pemovska T, Doornebal CW, Speksnijder EN, Wartha K, Jonkers J, de Visser KE. Development of metastatic HER2(+) breast cancer is independent of the adaptive immune system. *J Pathol* 2011; 224:56-66; PMID:21480230; <http://dx.doi.org/10.1002/path.2837>
- Vacchelli E, Senovilla L, Eggermont A, Fridman WH, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Chemotherapy with immunogenic cell death inducers. *Oncoimmunology* 2013; 2:e23510; PMID:23687621; <http://dx.doi.org/10.4161/onci.23510>
- Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013; 31:51-72; PMID:23157435; <http://dx.doi.org/10.1146/annurev-immunol-032712-100008>
- Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. *Immunity* 2013; 39:74-88; PMID:23890065; <http://dx.doi.org/10.1016/j.immuni.2013.06.014>

The immune system appears to play a critical role in the response to other targeted anticancer agents. For instance, by employing two distinct murine models of resistance to trastuzumab (an HER2-specific antibody currently approved for use in HER2<sup>+</sup> breast cancer patients)<sup>19-21</sup> induced by the loss of phosphatase and tensin homolog (*Pten*), the research team lead by Dihua Yu at the MD Anderson Cancer Center has recently demonstrated that the combination of a HER2-specific antibody other than trastuzumab and the AKT1 inhibitor triciribine effectively inhibits tumor growth in a T cell-dependent manner.<sup>22</sup> Indeed, besides blocking phosphoinositide-3-kinase (PI3K)/AKT1 and mitogen-activate protein kinase (MAPK) signaling, this combinatorial regimen turned out to promote the recruitment of CD4<sup>+</sup> and CD8<sup>+</sup> T cells to the tumor microenvironment, resulting in the elicitation a T<sub>H</sub>1 immune response against malignant cells. In line with this notion, interferon  $\gamma$  (IFN $\gamma$ )-neutralizing antibodies compromised the synergistic antitumor effects mediated by HER2-targeting antibodies plus triciribine.<sup>22</sup> Moreover, this immunochemotherapeutic regimen<sup>23</sup> increased the expression of

cytotoxic T lymphocyte-associated protein 4 (CTLA4, also known as CD152), a T-cell receptor that antagonizes the co-stimulatory activity of CD28,<sup>24</sup> and blocking CTLA4 (with a specific monoclonal antibody) further boosted the therapeutic potential of HER2-inhibiting antibodies combined with triciribine.<sup>22</sup>

An ideological debate on the preferability of transplantable vs. spontaneous mouse tumor models is sterile. Rather, the discussion should focus on what kind of model would reflect most accurately the pathophysiology and pharmacology of human (breast) carcinoma. Accumulating evidence indicates that the density, composition and function of the T-cell infiltrate has a major impact on the prognosis and therapeutic response of human breast cancers of different subtypes.<sup>5,7,25-29</sup> Based on these findings, it should be an obligation to use mouse models of breast cancer in which components of the immune system, in particular CD8<sup>+</sup> T lymphocytes, play a positive and decisive role.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

- Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, Portela Catani JP, Hannani D, Duret H, Steegh K, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* 2013; 38:729-41; PMID:23562161; <http://dx.doi.org/10.1016/j.immuni.2013.03.003>
- Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, Galluzzi L, Adjemian S, Kepp O, Niso-Santano M, et al. An immunosurveillance mechanism controls cancer cell ploidy. *Science* 2012; 337:1678-84; PMID:23019653; <http://dx.doi.org/10.1126/science.1224922>
- Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* 2011; 334:1573-7; PMID:22174255; <http://dx.doi.org/10.1126/science.1208347>
- Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat Rev Clin Oncol* 2011; 8:151-60; PMID:21364688; <http://dx.doi.org/10.1038/nrclinonc.2010.223>
- Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. *Nat Rev Drug Discov* 2012; 11:215-33; PMID:22301798; <http://dx.doi.org/10.1038/nrd3626>
- Zitvogel L, Apetoh L, Ghiringhelli F, André F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? *J Clin Invest* 2008; 118:1991-2001; PMID:18523649; <http://dx.doi.org/10.1172/JCI35180>
- Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* 2005; 11:312-9; PMID:15711557; <http://dx.doi.org/10.1038/nm1196>
- Park JM, Terabe M, Donaldson DD, Forni G, Berzofsky JA. Natural immunosurveillance against spontaneous, autochthonous breast cancers revealed and enhanced by blockade of IL-13-mediated negative regulation. *Cancer Immunol Immunother* 2008; 57:907-12; PMID:18004566; <http://dx.doi.org/10.1007/s00262-007-0414-0>
- Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 2010; 464:302-5; PMID:20220849; <http://dx.doi.org/10.1038/nature08782>
- Bos PD, Pliatas G, Rudra D, Lee SY, Rudensky AY. Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy. *J Exp Med* 2013; 210:2435-66; PMID:24127486; <http://dx.doi.org/10.1084/jem.20130762>
- Hannedóttir L, Tymoszuk P, Parajuli N, Wasmer MH, Philipp S, Daschil N, Datta S, Koller JB, Tripp CH, Stoitzner P, et al. Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response. *Eur J Immunol* 2013; 43:2718-29; PMID:23843024; <http://dx.doi.org/10.1002/eji.201242505>

17. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, Sattar H, Wang Y, Brown NK, Greene M, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell* 2010; 18:160-70; PMID:20708157; <http://dx.doi.org/10.1016/j.ccr.2010.06.014>
18. Stagg J, Loi S, Divisekera U, Ngiew SF, Duret H, Yagita H, Teng MW, Smyth MJ. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci U S A* 2011; 108:7142-7; PMID:21482773; <http://dx.doi.org/10.1073/pnas.1016569108>
19. Mavilio D, Galluzzi L, Lugli E. Novel multifunctional antibody approved for the treatment of breast cancer. *Oncoimmunology* 2013; 2:e24567; PMID:23802090; <http://dx.doi.org/10.4161/onci.24567>
20. Vacchelli E, Eggermont A, Galon J, Sautès-Fridman C, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Monoclonal antibodies in cancer therapy. *Oncoimmunology* 2013; 2:e22789; PMID:23482847; <http://dx.doi.org/10.4161/onci.22789>
21. Galluzzi L, Vacchelli E, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Zucman-Rossi J, Zitvogel L, Kroemer G. Trial Watch: Monoclonal antibodies in cancer therapy. *Oncoimmunology* 2012; 1:28-37; PMID:22720209; <http://dx.doi.org/10.4161/onci.1.1.17938>
22. Wang Q, Li SH, Wang H, Xiao Y, Sahin O, Brady SW, Li P, Ge H, Jaffee EM, Muller WJ, et al. Concomitant targeting of tumor cells and induction of T-cell response synergizes to effectively inhibit trastuzumab-resistant breast cancer. *Cancer Res* 2012; 72:4417-28; PMID:22773664; <http://dx.doi.org/10.1158/0008-5472.CAN-12-1339-T>
23. Vacchelli E, Prada N, Kepp O, Galluzzi L. Current trends of anticancer immunochemotherapy. *Oncoimmunology* 2013; 2:e25396; PMID:23894726; <http://dx.doi.org/10.4161/onci.25396>
24. Walker LS, Sansom DM. The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. *Nat Rev Immunol* 2011; 11:852-63; PMID:22116087; <http://dx.doi.org/10.1038/nri3108>
25. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov* 2011; 1:54-67; PMID:22039576; <http://dx.doi.org/10.1158/2159-8274.CD-10-0028>
26. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2010; 28:105-13; PMID:19917869; <http://dx.doi.org/10.1200/JCO.2009.23.7370>
27. Loi S. Tumor-infiltrating lymphocytes, breast cancer subtypes and therapeutic efficacy. *Oncoimmunology* 2013; 2:e24720; PMID:24073365; <http://dx.doi.org/10.4161/onci.24720>
28. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, Rouas G, Francis P, Crown JP, Hitre E, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol* 2013; 31:860-7; PMID:23341518; <http://dx.doi.org/10.1200/JCO.2011.41.0902>
29. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, Sautès-Fridman C, Ma Y, Tartour E, Zitvogel L, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. *Oncoimmunology* 2012; 1:1323-43; PMID:23243596; <http://dx.doi.org/10.4161/onci.22009>