

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

ErbB2 transgenic mice: a tool for investigation of the immune prevention and treatment of mammary carcinomas

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1903874> since 2023-05-16T13:47:57Z

Publisher:

John Wiley & Sons, Inc

Published version:

DOI:10.1002/0471142735.im2009s82

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

ErbB2 transgenic mice: a tool for investigation of the immune prevention and treatment of mammary carcinomas

Elena Quaglino, Cristina Mastini, Guido Forni and Federica Cavallo

Molecular Biotechnology Center, Department of Clinical and Biological Sciences,
University of Torino, Italy

Correspondence: elena.quaglino@unito.it

Transplantable versus transgenic mouse cancer models

During the last 20 years, a large assortment of tumors have been transplanted into syngeneic mice variously immunized and selectively immunosuppressed. Those transplanted in syngeneic mice form an attractive model because they grow quickly and provide prompt and clear-cut answers (Cavallo et al., 2006). Their growth and inhibition, in fact, can be assayed in weeks. Since transplantable tumors are commonly grafted in sites that are not the primary site of origin of human cancer, their relationship with their microenvironment is very different from that of human tumors and autochthonous mouse tumors. Models in which the tumor is transplanted orthotopically (i.e in the organ from which the tumor originated) are thought to more closely mimic the tumor-host interrelationships that characterize natural tumors (Kamb, 2005). Here, however, tumor cells are transplanted in a healthy organ where shortly start to grow, a situation that is a far cry from the long-standing relationship between a mutated cell and both its host's tissues

and the immune system that shapes the slow progression of natural carcinogenesis. In addition, the rapid growth kinetics of transplantable tumors minimizes the consequences of the genetic instability of natural tumors that leads to the immune selection of antigen-loss clones, a typical feature of natural carcinogenesis (Cavallo et al., 2005). Lastly, in young and healthy mice the immune system is not compromised by a long-lasting interaction with the incipient tumor. This is why they react much more effectively against a transplanted as opposed to an autochthonous tumor (Ostrand-Rosenberg, 2004).

Tumors arising in genetically engineered mice (GEM) provide an interesting alternative to transplantable tumor models in the study of human cancer since they recapitulate the stepwise progression of human cancer (Green and Hudson, 2005). In GEM, the relationship between the tumor, the surrounding tissues and the host immune system is fully preserved. Tumors grow slowly and may give rise to spontaneous metastases during their progression (Hüsemann et al., 2008). These features recapitulate those of human cancer much better than transplantable tumors and hence the information they provide is a surer guide in the extrapolation of data obtained in mice to the human setting. Moreover, GEM tolerance of the transgene-encoded protein is often comparable to human tolerance of tumor antigens (Lollini et al., 2005).

These advantages of GEM models, however, are offset by several drawbacks. The experiments are time consuming and may last more than one year. In some models, tumors are due to a gene aberration foreign to the human setting that leads to a pathogenic alteration with no exact human equivalent. The choice of the promoter inserted into the GEM genome drives the pattern of transgene expression and the timing of its first expression dramatically influences the kind and intensity of the immune tolerance of the transgene protein products. Lastly, cancers in GEM do not undergo the telomere crisis

responsible for many of the genomic aberrations in advanced human cancers. This is the result of the presence of long telomers and promiscuous telomerase in mice (Cavallo et al., 2006).

The information provided by GEM models is thus exposed subtle interpretation pitfalls, while their realistic modeling of several features of human tumors often leads to underestimation of its distortion. The complementation of admittedly poorly realistic but highly analytic transplantable tumor models with GEM tumor models could provide a more balanced assessment of the efficacy of the immune prevention and management of human cancer.

The *ErbB2* oncogene

Here the human orthologue of the *ErbB2* oncogene is referred as *Her-2* (human *ErbB-2*) while the rat orthologue is referred as *neu* since it was first isolated from a chemically induced rat neuroblastoma (Shih et al., 1981). The protein product coded by *ErbB2* gene (p185^{neu}) is a member of the epidermal growth factor receptor (EGFR) family with an intrinsic tyrosine kinase activity (King et al., 1985). Since it does not bind a specific ligand, activation of intracellular signal transduction is triggered by the formation of ligand-independent homodimers when it is mutated or ligand-dependent heterodimers with other EGFRs members when it is overexpressed (Garret et al. 2003).

ErbB2 is involved in normal physiological processes, such as embryogenesis, cell proliferation, differentiation, adhesion motility and apoptosis, while its malfunction or overexpression are responsible for development defects, diabetes and cancer.

Overexpression of Her-2 receptors, mainly due to gene amplification, is observed in 20-30% of human breast cancer and may be a poor prognosis marker (Ursini-Siegel et al.,

2007). Expression of an alternative splicing form of the *Her-2* oncogene (*delta Her-2*) corresponding to an in-frame deletion of an exon 16 amino acids long in the extracellular domain has also been reported in human breast cancer (Kwong and Hung, 1998). This form is constitutively active in a ligand-independent way as shown by its formation of disulphide-bridged homodimers (Ursini-Siegel et al., 2007; Kwong and Hung, 1998). Its expression level in human tumors is very low (about 5% of the Her-2 receptor), though it may contribute to mammary cancer progression by being responsible for the activating signals derived from homo dimerization (Ursini-Siegel et al., 2007). Deletion mutations in the corresponding region are involved in the formation of mammary carcinomas in mice transgenic for the wild type (WT) rat *neu* oncogene (Siegel et al., 1999). Moreover, comparative genomic hybridization/bacterial artificial chromosome analysis has shown that chromosomal deletions and amplifications are very frequent in Her-2⁺ human breast cancer and lead to regulation of oncogenes and tumor suppressor genes such as *Her-2* (Chin et al., 2006).

ErbB2's direct involvement in tumor pathogenesis makes it an ideal oncoantigen since it has a causal role in the promotion of tumor progression and is poorly expressed by normal tissue in adult humans and mice (Cavallo et al., 2007). Combination of chemotherapy with the anti-Her-2 monoclonal antibody, Trastuzumab (Herceptin) enhances anti-tumor activity in patients with advanced Her-2⁺ breast cancer (Marty et al., 2005). However, additional ways of improving clinical outcome are needed in order to achieve more persistent remissions and treatments that improve overall survival as opposed to that of a subset of patients.

As *ErbB-2* is directly involved in carcinogenesis, mice transgenic for the rat *neu* oncogene allow straightforward assessment of the ability of drugs and vaccines to inhibit the

progression of *neu*-driven cancer. Information from this model may provide indications on the efficacy of similar treatments in patients (Lollini et al., 2005).

Mice transgenic for *neu* oncogene

In view of the causal correlation between ErbB2 receptor overexpression and mammary carcinogenesis in humans and rats, several laboratories have assessed the tumorigenic potential of *ErbB2* in the mammary epithelium of transgenic mice in the last 20 years.

Transgenic mouse models that overexpress ErbB2 in their mammary epithelium have been generated, and it has been shown that overexpression or mutation of this receptor is crucial for the induction of breast cancer (Ursini-Siegel et al., 2007).

The first transgenic mouse model to express an activated form of rat *neu* (*neuT*) under the transcriptional control of the mouse mammary tumor virus promoter long terminal repeats (MMTV-LTR promoter) was generated in 1988 in Leder's laboratory (Muller et al., 1988).

The single-point mutation at position 664 in the transmembrane domain of neuT receptor leads to replacement of valine with glutamic acid, resulting in the formation of an H-bond with an alanine at position 661 of another neuT receptor, as well as with other EGFRs (Bargmann et al., 1986). These homo- and heterodimers spontaneously transduce the proliferative signals responsible for the transformation of mammary epithelial cells.

neuT transgenic female mice were first generated on the H-2^q FVB background (FVB-*neuT*). Mammary tumor progression of FVB-*neuT* females shows a latency time of about three months with occasional progression to pulmonary metastases. Mammary carcinomas arise almost synchronously, are polyclonal in origin and their cells are neuT⁺ (Muller et al., 1988).

Generation of these transgenic mice has shown that the *neuT* oncogene is sufficient to

induce murine mammary cancer. Apparently similar activated forms of *Her-2*, however, were not found in *Her-2*⁺ human breast tumors. These, in fact, mostly overexpress the WT *Her-2* proto-oncogene (neuN mice) (Ursini-Siegel, 2007).

To better mimic the human situation FVB mice transgenic for the *neuN* under the transcriptional control of the MMTV-LTR promoter were also established in the laboratory of Muller. Adult FVB-neuN transgenic female mice developed mammary carcinomas with a long latency ranging from 38 to 49 weeks. The cumulative number of independent tumors (tumor multiplicity), however, was only 2.6 tumors/mouse (Guy et al., 1992).

Recent studies have reported that in these mice, which are transgenic for the WT *neuN*, development of mammary cancer is mostly linked to activation of neu protein though point mutation and in-frame deletion of the oncogene sequence (Chan et al., 1999). Apart from the very long experimental times that would be required to assess the antitumoral efficacy of a drug or vaccine with this model, therefore, it does not provide an accurate representation of the human pathological situation.

BALB-neuT mice

To obtain *neuT* transgenic mice on the BALB/c genetic background more commonly used in immunological studies, we generated BALB-neuT mice starting from a random-bred progenitor transgenic for Muller's plasmid coding for the *neuT* oncogene (Lucchini et al., 1992). This mouse was mated with WT BALB/c females, and their *neuT*⁺ male offspring were backcrossed for more than 42 generations with regular BALB/c females. This mating provided the inbred BALB-neuT mice in which mammary tumor penetrance is 100%. All BALB-neuT female develop at least one palpable tumor within 5 months. Moreover, a progressively growing tumor is palpable in all their ten mammary glands within 7 months

(Boggio et al., 1998). These carcinomas overexpress neuT receptor and large lung metastases are evident during the late stages of tumor growth. Interestingly, single metastatic cells are already present in the lungs and bone marrow at a time when mammary samples showed only signs of noninvasive, atypical hyperplasia. Electron microscopy revealed microinvasion of stem-like cells and disseminated cancer cells in bone marrow expressing distinct stem cell markers. It would thus appear that systemic metastatic spread is an early event in BALB-neuT mammary carcinogenesis, as in several cases in human mammary cancer (Hüsemann et al., 2008).

Mammary carcinogenesis in BALB-neuT mice displays a very consistent stepwise and directly age-related progression that mimics several features of human breast carcinoma. Around the nipple of 4-week-old puberal mice, neuT positive cells give rise to epithelial nodular neoformations (side buds) protruding from the mammary ducts. These buds are foci of atypical hyperplasia in carcinomatous progression (Di Carlo et al., 1999). From week 4 to week 6, hyperplastic mammary epithelial cell proliferation is associated with an increase in the numbers of microvessels. This marked angiogenic switch is associated with the passage from *in situ* lesions to invasive cancer (Calogero et al., 2007). The side buds become more numerous and larger. Between the 10th and the 20th week, they become invasive and large metastases appear in the bone marrow and lungs. This progression is similar, albeit asynchronous, in all the mammary glands. All the cells of the hyperplastic, neoplastic and metastatic lesions greatly overexpress neuT protein in their cytoplasm and on their membrane (Boggio et al., 1998).

Since BALB-neuT mice display palpable tumors only four months after birth, their cells establish a long-lasting interaction with the microenvironment and thus mimic another important feature of human tumors. Carcinoma onset and progression are

straightforwardly driven by the *neuT* oncogene, whereas the early stages of the progression of mammary carcinogenesis are markedly modulated by inflammatory cytokines and factors released in the tumor microenvironment by infiltrating lymphocytes and the tumor cells themselves (Calogero et al., 2007). In contrast with the most common human breast cancers, mammary lesions are polyclonal and scattered all over the gland. However, neuT positive cell clones share the same initial mutation and their independent progression may resemble the clonal diversification that takes place in human cancer (Cavallo et al., 2006).

To assess the similarities between BALB-neuT and human mammary carcinomas we evaluated the pattern of gene expression associated with cancer progression and its microenvironment (Quaglino et al., 2004a). A subsequent comparison with the pattern in human Her-2 breast carcinomas revealed major similarities (Astolfi et al., 2005, Cavallo et al., 2007).

In addition, several features of BALB-neuT mice point to the acquisition of an immunological tolerance of neuT analogous to that of Her-2⁺ carcinomas patients.

Consistent with the *neuT* transgene expression in the thymus and its overexpression in the mammary gland, the immunoscope of the T cell repertoire of anti-neu immunized BALB-neuT mice showed the absence of the high-affinity anti-rat neu CD8⁺ T cell clones dominant in anti-neu immunized WT BALB/c mice (Rolla et al., 2006). Moreover, in BALB-neuT mice soluble factors released by tumor cells elicit an increase of immature myeloid cells in the peripheral blood and spleen. During the progression of the mammary lesions, this leads to a progressive inhibition of the immune response to alloantigens and to T cell proliferation triggered by anti CD3 monoclonal antibodies (Melani et al., 2003). As in cancer patients, in BALB-neuT mice progression of the mammary lesions is associated

with progressive expansion of the CD4⁺ CD25⁺, Foxp3⁺, GITR⁺ population of T regulatory (T_{reg}) cells. A few of them can be identified at the atypical hyperplasia stage of tumor progression, whereas they deeply infiltrate advanced tumors. T_{reg} cells hamper the anti neu immune reactivity naturally triggered by tumor progression and markedly limit vaccine efficacy. When they are removed by depleting BALB-neuT mice with anti CD25 monoclonal antibody, both antibodies and cytotoxic T-cell mediated natural immunosurveillance hamper the progression of neuT mammary carcinogenesis (Ambrosino et al., 2006).

Despite the numerous analogies, many features of mammary carcinogenesis in BALB-neuT mice are idiosyncratic. In the first place, the tumor is unleashed by a mutated oncogene inserted in a variable number of copies in the genome and in purely casual sites. Next, the expression of the oncogene is guided by a viral promoter that employs a transcription system which is completely different from that used by both the mouse and the human *ErbB2* endogenous promoter (Ursini- Siegel, 2007). To address this issue, a transgenic mouse model that places the *neuT* oncogene under the control of the endogenous mouse *ErbB2* promoter was generated in the Muller's laboratory (Andrechek et al., 2000). However, these new transgenic mice develop tumors that resemble the solid form of non-invasive human ductal carcinoma *in situ* (DCIS) in only one gland after a long latency period, and do not develop pulmonary metastases according with the histological features of DCIS (Andrechek et al., 2003). These drawbacks made this model virtually useless for immunological studies.

Prevention versus treatment of *neu* induced lesions

BALB-neuT mice are useful for evaluation of the protection afforded by drugs since they

inevitably develop lethal mammary carcinomas. The efficacy of an immunological or pharmacological treatment can be assessed by comparing the disease-free survival and the percentage of tumor-free mice in untreated versus treated mice over time. Moreover, as palpable mammary tumors occur in all ten mammary glands, increases in tumor size and tumor multiplicity are other parameters that can easily be recorded (Cavallo et al., 2006). The consistent stepwise progression of mammary carcinogenesis in all these mice allows evaluation of the potential of vaccines or drugs against each of its well-defined stages. It can also be determined they prevent the onset of tumors (prophylactic treatment) or inhibit their progression (therapeutic treatment) (Forni et al., 2003). In the first case, treatment should be started around the 6th week of age when mice display a diffuse atypical hyperplasia, in the second, from week 10 (multiple *in situ* carcinomas) to week 20 (invasive cancer), depending on the stage of the lesions to be treated. Previous data have demonstrated that the early stages of the aggressive progression in BALB-neuT females are hampered by stimulation of innate immunity (Cifaldi et al., 2001; Hayakawa et al., 2003). Much stronger protection is provided by *in vivo* electroporation of plasmids coding for the extracellular and transmembrane domains of the rat neu protein (Quaglino et al., 2004b). However, vaccination started when multifocal *in situ* carcinomas are already present does little to extend the disease-free survival time (Spadaro et al., 2004). Tumor prevention is thus an appropriate and rational goal for active immunity, whereas little protection is achieved when immunity is elicited in mice with diffuse lesions (Forni et al., 2003).

BALB-neuT mice knocked out (KO) for immunological related genes

Combination of spontaneous autochthonous mammary carcinogenesis of BALB-neuT

mice with the depletion of immunologically related genes permits investigation of both the mechanisms associated with natural immunosurveillance during tumor development in BALB-neuT mice and those associated with vaccine-induced prevention and treatment of *neu^t* mammary cancers.

Several BALB-neuT transgenic mice KO for genes related to immunological functions (here generically called BALB-neuT-KO mice) have been generated for this purpose, usually by crossing one BALB-neuT male with the KO female of interest and then by crossing *neuT⁺* F1 male mice again with the same KO female to obtain KO mice homozygous for the gene of interest and heterozygous for the *neuT* transgene.

Comparison of both the kinetics of tumor onset and the tumor multiplicity curves of BALB-neuT with BALB-neuT-KO mice illustrates the role of the KO gene in tumor development. Of the BALB-neuT-KO mice evaluated, those with the *interferon (IFN) γ* , *Fc γ RI/III* and *perforin (pfp)* genes KO display faster carcinogenesis. In untreated BALB-neuT/IFN γ -KO mice, carcinoma onset is significantly accelerated (Spadaro et al., 2004). This acceleration correlates with an enhanced tumor-induced angiogenesis that is no longer inhibited by the IFN γ naturally secreted (M. Iezzi, unpublished data). Mice KO for the gene coding for the *Fc receptor γ chain* do not express Fc γ RI/III, and thus lose natural killer (NK) cell-mediated, antibody-dependent cellular cytotoxicity (ADCC), opsonized phagocytosis by macrophages, and mast cell degranulation in response to FcR cross-linking (Takai et al., 1994). In untreated BALB-neuT/Fc γ RI/III-KO female mice, the tumor onset was significantly faster than in BALB-neuT mice, pointing to a substantial role of FcR-mediated natural effectors mechanisms in the control of *neuT* autochthonous carcinogenesis (unpublished data).

The importance of pfp in the inhibition of tumor onset is well known (Trapani and Smyth,

2002). When untreated BALB-neuT/pfp-KO mice were studied to assess the role of lymphocyte-mediated cytotoxicity in hampering *neuT*-driven carcinogenesis, it was evident that mammary carcinomas occurred earlier and in greater numbers compared to untreated BALB-neuT mice (Street et al., 2007). Once carcinomas develop, the effects mediated by pfp are lost, suggesting that lymphocyte-mediated cytotoxicity is only of importance in the control of the earliest stages of carcinogenesis (Street et al., 2007). The study of BALB-neuT mice KO for other immunologically related genes is instrumental for identification of key functions related to the inhibition and treatment of *neuT*-driven carcinomas in immunized BALB-neuT mice. Because selective depletion of immune functions through antibody administration is unsustainable in experiments lasting one year, variously KO BALB-neuT mice have been immunized and the protection afforded was compared with that of equally immunized BALB neuT mice. A decrease in protection points to the role of the KO gene. Vaccines of different kinds failed to inhibit *neuT*-driven carcinogenesis in BALB-neuT mice KO for the gene encoding the immunoglobulin μ chain (BALB-neuT/ μ -KO mice) who consequently do not produce immunoglobulins. The same inability of these vaccines to provide protection was observed in BALB-neuT/IFN γ -KO mice (Nanni et al., 2001, Quaglino et al., 2004b). These data show that the production of antibodies to rat neu and the secretion of IFN γ by immune cells are two crucial functions underlying the vaccine-induced inhibition of *neuT* lesions. Of the immune functions mediated by vaccine-induced antibodies, ADCC seems to have a prominent effector role. Indeed, sera from DNA-electroporated BALB-neuT mice guided a marked ADCC against neuT⁺ tumor cells (Quaglino et al., 2004b), whereas vaccine-induced protection is almost nil in BALB-neuT/Fc γ RI/III-KO mice (unpublished data). Lastly, dendritic cells (DC) are potent antigen-presenting cells fundamental in the

induction of T- and B-cell responses. The importance of lipid products derived from phosphoinositide 3-kinases (PI3K) in the ability of DC to reach sites of inflammation in response to chemotactic stimuli to obtain an optimal immune response has been demonstrated (Förster et al., 1999). Mice KO for the *PI3K γ* gene are viable and fertile, but their DC are less able to travel *in vivo* and mount T-cell-mediated specific immune responses in different experimental systems (Hirsch et al., 2000).

A strong inhibition of carcinogenesis was observed in BALB-neuT mice immunized through electroporation of plasmids encoding the extracellular and transmembrane domain of rat neuT. By contrast, no protection was evident in similarly immunized BALB-neuT mice KO for the *PI3K γ* gene (BALB-neuT/*PI3K γ* -KO mice). This result points to a key role of DC in DNA vaccination against neu.

Mice transgenic for the *Her-2* oncogene

Although *neu* transgenic mice provide an interesting model, the 10% difference in amino acid sequence between neu and Her-2 receptors suggests that central immune tolerance could be different in *neu* and *Her-2* transgenic mice since it may involve different peptides. The protective effect of the same vaccine could also be dissimilar against rat or human orthologues (Piechocki et al., 2003). Generation of transgenic mouse models expressing Her-2 may better predict the efficacy of vaccines against human Her-2⁺ mammary carcinomas. Mice transgenic for the *Her-2* oncogene under the transcriptional control of the MMTV-LTR generated in the laboratory of Groner did not develop mammary tumors, and they all died by 4 months of age as the result of preneoplastic kidney and lung lesions (Stocklin et al., 1993). C57BL/6 mice transgenic for *Her-2* gene under the transcriptional control of the whey acidic protein (WAP) promoter (B6-Her2) have since been generated

(Piechocki et al., 2003). B6-Her2 mice express high levels of Her-2 protein in their cerebellum and mammary tissue during pregnancy and lactation. Moreover, they tolerate Her-2 protein since they are unable to produce anti Her-2 antibodies as compared to their Her-2⁻ littermates, and the growth of a human Her-2⁺ line is faster than in Her-2⁻ littermates (Piechocki et al., 2003). Unfortunately B6-Her2 female mice do not develop spontaneous mammary carcinomas. However, they could be valuable for testing the protection afforded by anti Her-2 vaccines against transplantable Her-2⁺ tumors.

By crossing BALB-neuT males with B6-Her2 females, F1 mice (CB6F1, H-2^d/H-2^b) mice may express the *neu* transgene, and/or the *Her-2* transgene, or none. These four transgene combinations can be easily recognized by PCR genotyping. Thus, mice on the same genetic background display four transgene combinations and consequently four kinds of central tolerance of *neu* and *Her-2* oncogene protein products. These mice provide an interesting combination for evaluation of the efficacy of anti neu and anti Her-2 vaccines against transplantable neu⁺ or Her-2⁺ tumors and against autochthonous carcinomas arising in hosts tolerant of the *neu* transgene or the combination of *neu* and *Her-2* transgene. In effect, whereas CB6F1-Her2 females do not develop autochthonous mammary cancer (Figure 1 A and B), CB6F1-neuT/Her2 double transgenic female mice do develop neu⁺ mammary carcinomas with the same penetrance and tumor growth kinetics as CB6F1-neuT mice (Figure 2). The autochthonous carcinomas growing in *neu/Her-2* double transgenic mice are morphologically identical to those of CB6F1-neuT mice (Figure 1 C and D). However in *neu/Her-2* double transgenic mice Her-2 protein expression was undetectable in the growing tumors.

A transgenic mouse model of spontaneous mammary Her-2⁺ tumors is that generated in the laboratory of Erickson (Finkle et al., 2004). These mice are transgenic for the WT *Her-*

2 oncogene under the transcriptional control of the MMTV-LTR promoter and develop spontaneous mammary tumors with an incomplete penetrance: 76% of transgenic females develop few asynchronous but rapidly growing Her-2⁺ tumors with an average latency of about 28 weeks. Highly Her-2⁺ pulmonary metastases have been noted in only 23% of tumor-bearing mice and Her-2 protein expressed by independent tumors displays a unique mutation in the juxtamembranous region (Finkle et al., 2004).

All these transgenic mouse models could be used for evaluation of the efficacy of anti tumor strategies. A common issue, however, is that their tolerance of the transgenic ErbB2 protein may be very different from that of patients and healthy subjects against self ErbB2 (Lollini et al., 2005). To address this issue, a mouse model of breast cancer caused by expression of the polyoma middle T (PyMT) oncoprotein in the mammary epithelium (Lin et al., 2003) may be studied. PyMT is a membrane-attached protein encoded by the small DNA polyoma virus that acts as a potent oncogene. These transgenic mice provide a reliable model of spontaneous mammary tumors expressing a syngeneic mouse ErbB2 protein (Lin et al., 2003). Expression of the PyMT oncoprotein is under the control of the MMTV-LTR promoter and is therefore restricted to the mammary epithelium. *PyMT* transgenic mice display a high frequency of pulmonary metastases, 100% tumor penetrance, a short latency and pregnancy-independence (Guy et al., 1992). Moreover, tumor progression is stepwise and comparable to human breast diseases, though PyMT is not expressed in human breast tumor cells. However, the role played by the mouse *ErbB2* oncogene in the progression of these tumors and the consistency of its expression are issues that have to be defined.

In conclusion, all mouse models of ErbB2 mammary carcinogenesis generated so far may contribute to the development of immunological strategies designed to prevent and cure

Her-2+ breast tumors. Each model is subject to major limitations that hamper generalization of the experimental findings. This is particularly true as far as vaccines are concerned, since the kind of tolerance may drastically affect their efficacy. Epitopes of crucial importance in the immunogenicity of the neu receptor may be absent in Her-2 receptors. Lastly, antigen processing, peptide presentation by the major histocompatibility glycoproteins and self-tolerance may be very different from one model to the next, and thus carry significant weight in the clinical extrapolation of experimental information. Each of these features is a single variable. Elaboration of a perfect model accounting for all the variables, however, is an unlikely proposition. Integration and critical evaluation of the information provided by each model may thus prove the only feasible source of reliable predictions on the effect of individual treatments and vaccines in human patients.

Acknowledgements

This work was supported by grants from Italian Association for Cancer Research; the Italian Ministero dell'Università e della Ricerca; the University of Torino; the Compagnia di San Paolo, Torino; the Fondazione Denegri, Torino; the Regione Piemonte; and Nordic Centre of Excellence for development of anti tumour Vaccine concepts (NCEV). We thank Prof. John Iliffe for critical reading of the manuscript.

References

Ambrosino, E., Spadaro, M., Iezzi, M., Curcio, C., Forni, G., Musiani, P., Wei, W.Z. and Cavallo, F. 2006. Immunosurveillance of ErbB2 carcinogenesis in transgenic mice is concealed by a dominant regulatory T-cell self-tolerance. *Cancer Res.* 66:7734-7740.

Andrechek, E.R., Hardy, W.R., Siegel, P.M., Rudnicki, M.A., Cardiff, R.D. and Muller, W.J. 2000. Amplification of the neu/erbB-2 oncogene in a mouse model of mammary tumorigenesis. *Proc Natl Acad Sci U S A.* 97:3444-3449.

Andrechek, E.R., Laing, M.A., Girgis-Gabardo, A.A., Siegel, P.M., Cardiff, R.D. and Muller, W.J. 2003. Gene expression profiling of neu-induced mammary tumors from transgenic mice, reveals genetic and morphological similarities to ErbB2-expressing human breast cancers. *Cancer Res.* 63:4920-4926.

Astolfi, A., Landuzzi, L., Nicoletti, G., De Giovanni, C., Croci, S., Paladini, A., Ferrini, S., Iezzi, M., Musiani, P., Cavallo, F., Forni, G., Nanni, P. and Lollini, P.L. 2005. Gene expression analysis of immune-mediated arrest of tumorigenesis in a transgenic mouse model of HER-2/neu-positive basal-like mammary carcinoma. *Am J Pathol.* 166:1205-1216.

Bargmann, C.I., Hung, M.C., and Weinberg, R.A. 1986. Multiple independent activations of the neu oncogene by a point mutation altering the transmembrane domain of p185. *Cell.* 45:649-657.

Boggio, K., Nicoletti, G., Di Carlo, E., Cavallo, F., Landuzzi, L., Melani, C., Giovarelli, M., Rossi, I., Nanni, P., De Giovanni, C., Bouchard, P., Wolf, S., Modesti, A., Musiani, P., Lollini, P.L., Colombo, M.P. and Forni, G. 1998. Interleukin 12-mediated prevention of spontaneous mammary adenocarcinomas in two lines of Her-2/neu transgenic mice. *J Exp*

Med. 188:589-596.

Calogero, R.A., Corsero, F., Forni, G. and Cavallo, F. 2007. Inflammation and breast cancer. Inflammatory component of mammary carcinogenesis in ErbB2 transgenic mice. *Breast Cancer Res.* 9: 211

Cavallo, F., Curcio, C., and Forni, G. 2005. Immunotherapy and immunoprevention of cancer: where do we stand? *Expert Opin. Biol. Ther.* 5:717-726.

Cavallo, F., Offringa, R., van der Burg, S.H., Forni, G. and Melief, C.J. 2006. Vaccination for treatment and prevention of cancer in animal models. *Adv Immunol.* 90:175-213.

Cavallo, F., Calogero, R.A. and Forni, G. 2007. Are oncoantigens suitable targets for anti-tumour therapy? *Nat Rev Cancer.* 7:707-713.

Chan, R., Muller, W.J. and Siegel, P.M. 1999. Oncogenic activating mutations in the neu/erbB-2 oncogene are involved in the induction of mammary tumors. *Ann N Y Acad Sci.* 889:45-51.

Chin, K., DeVries, S., Fridlyand, J., Spellman, P.T., Roydasgupta, R., Kuo, W.L., Lapuk, A., Neve, R.M., Qian, Z., Ryder, T., Chen, F., Feiler, H., Tokuyasu, T., Kingsley, C., Dairkee, S., Meng, Z., Chew, K., Pinkel, D., Jain, A., Ljung, B.M., Esserman, L., Albertson, D.G., Waldman, F.M. and Gray, J.W. 2006. Genomic and transcriptional aberration linked to breast cancer pathophysiology. *Cancer Cell.* 10:529-541.

Cifaldi, L., Quaglino, E., Di Carlo, E., Musiani, P., Spadaro, M., Lollini, P.L., Wolf, S., Boggio, K., Forni, G. and Cavallo, F. 2001. A light, nontoxic interleukin 12 protocol inhibits HER-2/neu mammary carcinogenesis in BALB/c transgenic mice with established hyperplasia. *Cancer Res.* 61:2809-2812.

Di Carlo, E., Diodoro, M.G., Boggio, K., Modesti, A., Modesti, M., Nanni, P., Forni, G. and Musiani, P. 1999. Analysis of mammary carcinoma onset and progression in HER-2/neu oncogene transgenic mice reveals a lobular origin. *Lab Invest.* 1999 79:1261-1269.

Finkle, D., Quan, Z.R., Asghari, V., Kloss, J., Ghaboosi, N., Mai, E., Wong, W.L., Hollingshead, P., Schwall, R., Koeppen, H. and Erickson, S. 2004. HER2-targeted therapy reduces incidence and progression of midlife mammary tumors in female murine mammary tumor virus huHER2-transgenic mice. *Clin Cancer Res.* 10:2499-2511.

Forni, G., Curcio, C., Spadaio, M., Iliffe, J., Quaglino, E., Di Carlo, E., Musiani, P. and Lollini, P.L. 2003. Immunization in tumor prevention. *Int Immunopharmacol.* 3:1151-1158.

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E. and Lipp, M. 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell.* 99:23-33.

Garrett, T.P., McKern, N.M., Lou, M., Elleman, T.C., Adams, T.E., Lovrecz, G.O., Kofler, M., Jorissen, R.N., Nice, E.C., Burgess, A.W., and Ward, C.W. 2003. The crystal structure

of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol Cell*. 11:495-505.

Green, J.E. and Hudson, T. 2005. The promise of genetically engineered mice for cancer prevention studies. *Nat. Rev. Cancer*. 5:184-198.

Guy, C.T., Webster, M.A., Schaller, M., Parsons, T.J., Cardiff, R.D. and Muller, W.J. 1992. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci U S A*. 89:10578-10582.

Guy, C.T., Cardiff, R.D. and Muller, W.J. 1992. Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. *Mol Cell Biol*. 12:954-961.

Hayakawa, Y., Rovero, S., Forni, G. and Smyth, M.J. 2003. Alpha-galactosylceramide (KRN7000) suppression of chemical- and oncogene-dependent carcinogenesis. *Proc Natl Acad Sci U S A*. 100:9464-9469.

Hirsch, E., Katanaev, V.L., Garlanda, C., Azzolino, O., Pirola, L., Silengo, L., Sozzani, S., Mantovani, A., Altruda, F. and Wymann, M.P. 2000. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science*. 287:1049-1053.

Hüsemann, Y., Geigl, J.B., Schubert, F., Musiani, P., Meyer, M., Burghart, E., Forni, G., Eils, R., Fehm, T., Riethmüller, G. and Klein, C.A. 2008. Systemic spread is an early step

in breast cancer. *Cancer Cell*. In press.

Kamb, A. 2005. What's wrong with our cancer models? *Nat. Rev. Drug Discov.* 4:161-165.

King, C.R., Kraus, M.H. and Aaronson S.A. 1985. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. *Science.* 229:974-976.

Kwong, K.Y. and Hung, M.C. 1998. A novel splice variant of HER2 with increased transformation activity. *Mol. Carcinog.* 23:62-68.

Lin, E.Y., Jones, J.G., Li, P., Zhu, L., Whitney, K.D., Muller, W.J. and Pollard, J.W. 2003. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am J Pathol.* 163:2113-2126.

Lollini, P.L., Nicoletti, G., Landuzzi, L., De Giovanni, C. and Nanni, P. 2005. New target antigens for cancer immunoprevention. *Curr Cancer Drug Targets.* 5:221-228.

Lucchini, F., Sacco, M.G., Hu, N., Villa, A., Brown, J., Cesano, L., Mangiarini, L., Rindi, G., Kindl, S., Sessa F, Vezzoni, P. and Clerici, L. 1992. Early and multifocal tumors in breast, salivary, harderian and epididymal tissues developed in MMTY-Neu transgenic mice. *Cancer Lett.* 64:203-209.

Marty, M., Cognetti, F., Maraninchi, D., Snyder, R., Mauriac, L., Tubiana-Hulin, M., Chan, S., Grimes, D., Antón, A., Lluch, A., Kennedy, J., O'Byrne, K., Conte, P., Green, M., Ward,

C., Mayne, K. and Extra, J.M. 2005. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol.* 23:4265-4274.

Melani, C., Chiodoni, C., Forni, G. and Colombo, M.P. 2003. Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. *Blood.* 102:2138-2145.

Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R., and Leder, P. 1988. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell.* 54:105-115.

Nanni, P., et al., 2001. Combined allogeneic tumor cell vaccination and systemic IL12 prevents mammary carcinogenesis in HER-2/neu transgenic mice. *J. Exp. Med.* 194:1195-1205.

Ostrand-Rosenberg, S. 2004. Animal models of tumor immunity, immunotherapy and cancer vaccines. *Curr Opin Immunol.* 16:143-150.

Piechocki, M.P., Ho, Y.S., Pilon, S. and Wei, W.Z. 2003. Human ErbB-2 (Her-2) transgenic mice: a model system for testing Her-2 based vaccines. *J Immunol.* 171:5787-5794.

Quaglino, E., Rolla, S., Iezzi, M., Spadaio, M., Musiani, P., De Giovanni, C., Lollini, P.L.,

Lanzardo, S., Forni, G., Sanges, R., Crispi, S., De Luca, P., Calogero, R. and Cavallo, F. 2004a. Concordant morphologic and gene expression data show that a vaccine halt HER-2/neu preneoplastic lesions. *J Clin Invest.* 113:709-717.

Quaglino, E., Iezzi, M., Mastini, C., Amici, A., Pericle, F., Di Carlo, E., Pupa, S.M., De Giovanni, C., Spadaro, M., Curcio, C., Lollini, P.L., Musiani, P., Forni, G. and Cavallo F. 2004b. Electroporated DNA vaccine clears away multifocal mammary carcinomas in her-2/neu transgenic mice. *Cancer Res.* 64:2858-2864.

Rolla, S., Nicolás, C., Malinarich, S., Orsini, M., Forni, G., Cavallo, F. and Ria, F. 2006. Distinct and non-overlapping T cell receptor repertoires expanded by DNA vaccination in wild-type and HER-2 transgenic BALB/c mice. *J Immunol.* 177:7626-7633.

Shih, c., Padhy, L.C., Murray, M. And Weinberg, R.A. 1981. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. *Nature.* 290:261-264

Siegel, P.M., Ryan, E.D., Cardiff, R.D. and Muller, W.J. 1999. Elevated expression of activated forms of Neu/ErbB-2 and ErbB-3 are involved in the induction of mammary tumors in transgenic mice: implications for human breast cancer. *EMBO J.* 18:2149-2164.

Spadaro, M., Lanzardo, S., Curcio, C., Forni, G. and Cavallo, F. 2004. Immunological inhibition of carcinogenesis. *Cancer Immunol Immunother.* 53:204-216.

Stöcklin, E., Botteri, F. and Groner, B. 1993. An activated allele of the c-erbB-2 oncogene

impairs kidney and lung function and causes early death of transgenic mice. *J Cell Biol.* 122:199-208.

Street, SE., Zerafa, N., Iezzi, M., Westwood, JA., Stagg, J., Musiani, P. and Smyth, MJ. 2007. Host perforin reduces tumor number but does not increase survival in oncogene-driven mammary adenocarcinoma. *Cancer Res.* 67:5454-60

Takai, T., Li, M., Sylvestre, D., Clynes, R. and Ravetch, J.V. 1994. FcR gamma chain deletion results in pleiotrophic effector cell defects. *Cell.* 76:519-529.

Trapani, J.A. and Smyth, M.J. 2002. Functional significance of the perforin/granzyme cell death pathway. *Nat Rev Immunol.* 2:735-747.

Ursini-Siegel, J., Schade, B., Cardiff, R.D. and Muller, W.J. 2007. Insights from transgenic mouse models of ERBB2-induced breast cancer. *Nat. Rev. Cancer.* 7:389-397.

Caption to figures

Figure 1. Whole mount of the mammary glands from 22 weeks old wild-type CB6F1, CB6F1-Her2, CB6F1-neuT (C) and CB6F1-neuT/Her2 (D) female mice. The mammary glands of both wild-type CB6F1 (A), CB6F1-Her2 (B) mice are a tree-like duct structure originating from the nipple (N) and extending into the fat pad. By contrast, the mammary gland of CB6F1-neuT (C) and CB6F1-neuT/Her2 (D) transgenic mice shows neoplastic lesions (black arrows) ranging from atypical hyperplasia foci to *in situ* carcinomas. The central oval black areas (arrowhead) are mammary lymph nodes. Magnification x6.3.

Figure 2. Incidence of mammary carcinomas in untreated CB6F1-neuT (grey line, 8 mice) and CB6F1-neuT/Her2 (black line, 12 mice) transgenic mice. Percentage of tumor free mice are shown in function of time. Mice with at least one progressively growing mammary tumor > 1 mm mean diameter were classed as tumor-bearing mice.