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Evolution of animal models in cancer vaccine development

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Abstract

Advances in cancer vaccine development are facilitated by animal models reflecting key features of human cancer and its interface with host immunity. Several series of transplantable preneoplastic and neoplastic mouse mammary lesions have been used to delineate mechanisms of anti-tumor immunity. Mimicking immune tolerance to tumor-associated antigens (TAA) such as HER2/neu, transgenic mice developing spontaneous mammary tumors are strong model systems for pre-clinical vaccine testing. In these models, HER2 DNA vaccines are easily administered, well-tolerated, and induce both humoral and cellular immunity. Although engineered mouse strains have advanced cancer immunotherapy, basic shortcomings remain. For example, multiple mouse strains have to be tested to recapitulate genetic regulation of immune tolerance in humans. Outbred domestic felines more closely parallel humans in the natural development of HER2 positive breast cancer and their varying genetic background. Electrovaccination with heterologous HER2 DNA induces robust adaptive immune responses in cats. Importantly, homologous feline HER2 DNA with a single amino acid substitution elicits unique antibodies to feline mammary tumor cells, unlocking a new vaccine principle. As an alternative approach to targeted vaccination, non-surgical tumor ablation such as cryoablation induces anti-tumor immunity via *in situ* immunization, particularly when combined with toll-like receptor (TLR) agonist. As strategies for vaccination advance, non-invasive monitoring of host response becomes imperative. As an example, magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning following administration of tryptophan metabolism tracer [11C]-alpha-methyl-tryptophan (AMT) provides non-invasive imaging of both tumor growth and metabolic activities. Because AMT is a substrate of indoleamine-pyrrole 2,3-dioxygenase (IDO), an enzyme that produces the immune regulatory molecule kynurenine, AMT imaging can provide novel insight of host response. In conclusion, new feline models improve the predictive power of cancer immunotherapy and real-time PET imaging enables mechanistic monitoring of host immunity. Strategic utilization of these new tools will expedite cancer vaccine development.

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Keywords

Animal model; transgenic mouse; domestic cat; cancer vaccine; DNA vaccine; HER2/neu; cancer imaging; tumor ablation; in situ immunization

Introduction

To predict human response to cancer vaccines, candidate regimens should be interrogated in a fully functional immune system. To date, only intact animals carrying all immunological constituents can mimic human responses to vaccination or associated immune modulation. Therefore, the choice of animal models is critical in cancer vaccine research. Comparative oncology models where animal malignancies express analogous tumor-associated antigens (TAA) and exhibit disease progression similar to humans may provide greater predictive power of treatment outcomes. Artificial or foreign antigens frequently used in vaccine studies may induce striking reactivity in pre-clinical models (e.g. ovalbumin), but the findings often fall short in predicting patient response. Even genetically modified inbred mice with spontaneous tumor development may not fully recapitulate human tumor immunology. These pre-clinical model inadequacies may have hindered clinical translation of cancer vaccines. At a time when cancer immunotherapy is coming to the forefront, it is opportune to re-examine the usage of animal models. Rather than conducting a comprehensive review of all models ever tested, we will discuss animal models and mammary tumor systems with a focus on HER2/neu antigen to show the utility, futility and new development of animal models.

Early animal models and tumor cell lines

Early studies of mammary tumors in syngeneic mice captured many biological parallels between human breast cancer and spontaneous mouse mammary tumors. The cell lines derived from these tumors continue to be strong tools in cancer immunotherapy research. In the 1960s and 1970s, mouse mammary tumor virus (MMTV) [1;2], hormones and chemical carcinogens [3–5] were used as experimental tumor inducing agents (Fig. 1, left panel). MMTV induces mammary tumors by insertional activation of oncogenes or genes critical in tumorigenesis [6;7]. A number of MMTV activated genes are also deregulated and/or mutated in primary human breast cancer, suggesting parallel biology between breast cancer and MMTV induced tumors [6;8]. Infectious MMTV particles can be introduced into any mouse strain through virally contaminated milk [9]. For example, BALB/cF3H (B6C3H) mice were derived from BALB/c pups fostered by MMTV infected C3H mothers. The pups were infected via nursing and ultimately went on to develop mammary tumors. In MMTV induced primary tumors or tumor cell lines, MMTV associated antigens are often down-regulated to avoid immune recognition, but when expression is exogenously induced they become potent foreign antigens in wild type mice.

From a single MMTV induced mouse mammary tumor in a B6C3H mouse, a panel of tumor cell lines were derived and characterized by our group [10;11]. MMT lines 66, 67, 68H, 168 and 4.10 were in the initial panel. Of these lines, only 68H expressed significant levels of MMTV envelop protein antigens, although such expression can be induced in the other lines

[12]. Through *in vivo* selection for metastatic behavior and *in vitro* selection for ouabain and/or thioguanine resistance, two highly metastatic lines 4T07 and 4T1 were established from 4.10. Selection for ouabain or thioguanine resistance was primarily for research convenience (e.g. enumeration of tumor cells after culturing with test drugs). The thioguanine resistant line 4T1 has been used in numerous tumor metastasis studies [13]. 4T1 tumors produce large quantities of myeloid cell stimulating cytokines, which cause tumor-bearing mice to develop splenomegaly with heavy myeloid cell infiltration. Many studies of myeloid derived suppressor cells (MDSC) have been conducted with the 4T1 model, where myeloid cell expansion may be exaggerated. Still, 4T1 and its sister MMT lines are powerful tools in cancer research, especially when experimental results are interpreted with consideration of their derivation history [11].

Another series of mammary tumor cell lines were derived from tumors that arose in serially passaged, preneoplastic mammary hyperplastic alveolar nodules (HAN) that developed in a BALB/c female mouse with uninhibited prolactin secretion [3;4]. HAN tissue can be serially passaged in cleared (i.e. surgical removal of host mammary gland) mammary fat pads of 3 week old females. This tissue maintains the hyperplastic alveolar morphology for several months until spontaneous tumors arise from it. From these spontaneous tumors, MMT lines D2F2 and D2A1 were established [14]. Although these lines are not infected with milk-transmitted MMTV, nor selected with drugs, spectral karyotyping analysis showed a number of chromosomal aberrations, suggesting genetic alterations as a result of tumorigenesis and cell line derivation [15]. The D2F2 line and its derivatives transfected to express oncogenes or TAAs are frequently used in our and others' studies [11;16–21].

Rat neu transgenic (Tg) mice

When genetic engineering of mice became possible, mice expressing human TAA were generated to enable the study of immune reactivity or tolerance to TAA. Tg mice expressing rat neu oncogene under the MMTV promoter have been generated in several genetic backgrounds [22]. Among them, BALB NeuT (NeuT) mice are BALB/c mice expressing a transforming rat neu gene (Fig. 1, middle, bottom panel) [23;24]. These mice are maintained as heterozygotes by breeding with BALB/c mice, as homozygotes are not viable. All NeuT females develop up to 10 spontaneous mammary tumors, one from each mammary gland, around the age of 17–19 weeks. NeuT mice show immune tolerance and reduced response to rat neu immunization as reported by us and others [18;25–29]. Several neu positive mammary tumor lines have been established from NeuT spontaneous tumors such as TUBO [30], Bam1a and BamIR5 [31], which grow progressively in both NeuT and wild type BALB/c mice. TUBO and Bam1a tumors are highly dependent on the HER2/neu signaling pathway for their survival and are very sensitive to the cytotoxic effect of tyrosine kinase inhibitors or anti-neu antibodies [18;31;32]. BamIR5 cells were selected in culture with the EGFR inhibitor gefitinib. In contrast to TUBO or BAM1a cells, D2F2/neu cells transfected to express rat neu are resistant to anti-neu antibody, but sensitive to T-cell cytotoxicity. D2F2/neu represents an antibody resistant HER2/neu positive tumor cell line [15]. Together, wild type BALB/c and NeuT mice, with tumor lines TUBO, BAM1a, BAM IR5, D2F2 and D2F2/neu constitute a comprehensive test system to investigate neu related immune responses, neu targeted vaccine efficacy and other immunotherapy approaches.

Another strain of rat neu Tg mice, FVB/N-Tg(MMTVneu)202Mul/J (FVB/N), express wild type neu under the MMTV promoter/enhancer [33]. Approximately half of the females develop spontaneous mammary tumors around the age of 28 weeks [33]. FVB/N mice have also been used extensively in HER2/neu vaccination studies that target rat neu [34–36]. However, the most relevant animal model for studying immune tolerance to rat neu remains the wild type rat, which exhibits natural tolerance to rat neu. Disis et al. showed the induction of T-cell immunity against rat neu in rats using heterologous human HER2 protein [37] or rat neu peptides [38]. Following this concept, a number of human clinical trials were conducted with HER2 peptides [39;40](<http://clinicaltrials.gov>). Reactivity to HER2 peptides was found in human HER2 DNA vaccinated breast cancer patients [41], illustrating the feasibility of eliciting immune reactivity to self HER2.

Human HER2 Tg mice

Using the whey acidic protein (WAP) promoter, which is functional in mammary epithelial cells of pregnant or lactating mice, we generated HER2 Tg mice in both C57BL/6 (B6) (B6.Cg-Tg(Wap-ERBB2)229Wzw/J, Jackson laboratory) and BALB/c (BALB) backgrounds [42;43]. Immune tolerant status to human HER2 was surprisingly disparate, with BALB HER2 Tg mice showing >10 fold greater antibody and T-cell responses than their B6 counterparts when vaccinated with HER2 [16;17;42] (Fig. 1, middle top panel). The F1 offspring between the two strains had an intermediate response. This differential response to vaccination against HER2 suggests MHC haplotype or other genetic control of immune reactivity to tumor-associated self-antigens. Each syngeneic mouse strain would therefore represent a single individual in a genetically diverse population.

Human HLA-DRB1*0301 (DR3), that renders susceptibility to autoimmune diseases was introduced into B6 HER2 Tg mice to evaluate MHC regulation of self-reactivity [44;45]. Compared to B6 HER2 Tg mice, (C57BL/6xHLA-DR3)F1 HER2 Tg mice developed more severe autoimmune thyroiditis when immunized with thyroglobulin and produced 3 fold higher α -HER2 antibody when immunized with HER2 positive human ovarian cancer line SKOV3 [32], again showing genetic regulation of immune response to TAA. Negative regulation of immune reactivity can also be attributed to regulatory cells such as CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Treg). Depletion of Tregs in highly tolerant B6 Her2 Tg mice released some negative regulation to increase vaccine response, supporting the role of Tregs in immune tolerance [17;32]. Overall, vaccine response in HER2 Tg mice follows the genetic pattern of BALB > (BALBxB6) F1 > (B6xDR3) F1 > B6. Based on the disparate HER2 vaccine response between strains, one must question which strain is more representative of human immunology and how many strains should be tested in pre-clinical studies. It would seem that human patients can only be mimicked by an assortment of transgenic mice with different genetic backgrounds, or using a non-syngeneic system with inherent genetic heterogeneity.

Tg mice expressing multiple human antigens and in multiple genetic backgrounds would be the next logical step for vaccine testing. Using a bicistronic carcinoembryonic antigen (CEA)/HER2 DNA vaccine (V932), we showed reactivity to both antigens in HER2/CEA dual Tg mice [46]. The feasibility of dual targeted DNA vaccines is encouraging and may

lead to the advent of multi-targeted vaccines. Although Tg mice remain an essential component in vaccine development, there are limitations. In Tg mice, exogenous promoters are often used to drive TAA, with mouse rather than human MHC presenting the antigenic peptides. T-cell repertoires are selected in the thymus where both mouse and human antigens are presented, further confounding the system. The short life span, lack of natural tumorigenesis and comparatively small body weight relative to surface area are just a few more disparities between rodents and humans. Some caveats of laboratory mice may further constrain vaccine research, such as the expression of superantigen from endogenous MMTV long terminal repeat in nearly all laboratory mouse strains, which activates and deletes subpopulations of T cells as we reported [47;48]. Thus, it is prudent to consider alternative systems that more naturally mimic humans in both cancer development and immune function.

Canine and feline mammary tumor models

Domestic dogs and cats share living environments with their owners and develop mammary cancers similar to humans. Dogs develop mammary tumors more frequently [49;50], but half of all canine mammary tumors are benign, while most feline mammary tumors are malignant adenocarcinomas [51]. Hormonal regulation of feline mammary carcinoma (FMC) is evident because tumor incidence is drastically reduced by ovariectomy [50;52]. Disease progression and histopathology of FMC are similar to that of human breast cancer [51;53–60]. FMC express EGFR, HER2 and HER3 as we reported with two feline mammary tumor lines K12 [61] and K248 [62] as well as primary tumor tissue [63]. ERBB family receptors are functional in these cell lines, as evident from the receptor tyrosine kinase inhibitors (TKI) gefitinib and lapatinib reducing proliferation *in vitro* and HER2 and AKT phosphorylation detected in primary tumor samples by Western blot. The similarities between human breast cancer and FMC are striking, such that cats may be a valuable model for human breast cancer.

DNA vaccines have advanced more expediently in veterinary than human medicine. The first clinically approved therapeutic cancer vaccine in any species is a plasmid DNA encoding human tyrosinase for canine oral melanoma [51;64;65]. Two other DNA vaccines targeting West Nile virus in horses [66] and hematopoietic necrosis virus in salmon [67] have also been approved for veterinary and agricultural use, respectively. Vaccination of canine oral melanoma patients with human chondroitin sulfate proteoglycan-4 (CSPG-4) DNA induced specific immunity [68]. In dogs with non-Hodgkin's lymphoma, CD40-B cells transfected with autologous tumor RNA stimulated tumor immunity [69]. We propose that outbred domestic cats and dogs are an effective intermediate test system for cancer vaccines, and represent a genetically heterogeneous population that is more capable of predicting the range of immune responses in humans. The findings will expedite human vaccine development while benefiting feline and canine cancer patients.

Evolution of HER2 DNA vaccines

Vaccine design can be further developed and appropriately assessed for transition to humans once relevant model systems are in place. Our lab was the first to report a panel of kinase

defective HER2 constructs encoding recombinant proteins that are directed to different subcellular compartments. The full-length kinase-null pE2A (pE2A-K735) induced HER2 antibody and T-cell responses in wild type and BALB HER2 Tg mice and in breast cancer patients (Fig. 2, left panel) [21;41]. The pE2TM construct has similar activity, but only encodes the HER2 extracellular and transmembrane domains. Vaccine response to psecE2 encoding a secreted protein was equivalent to that of pE2A and pE2TM, while cytosol-restricted pcytE2 induced only CD8 T-cell responses. Broadening of epitope recognition to other TAA after HER2 vaccination was also observed and illustrates the ability to promote *in situ* priming after vaccine-induced tumor cell destruction [20]. These designs have been adapted and optimized to generate a vaccine for two clinical trials [46;70;71] which showed safety. However, HER2 T-cell response induced in one trial was limited, possibly because the vaccine was combined with CEA DNA fused to a strong foreign antigen, thus skewing immune reactivity away from HER2. The efficacy of DNA-based vaccination has steadily improved following a series of innovations, including co-vaccination with immunostimulatory molecules, electroporation to amplify DNA delivery [15;17], immune modulation (e.g. depletion of Tregs) and modification of antigen DNA sequences [16;72].

In several experimental autoimmune models, tolerance to self-antigens is overcome by immunization with heterologous (xenogeneic) antigens. Murine experimental autoimmune encephalomyelitis is induced by bovine or guinea pig myelin basic protein [73;74]. In murine collagen-induced arthritis, autoimmunity is induced by bovine, porcine, or human collagen [75]. Immunization of mice with the *Torpedo californica* acetylcholine receptor induces experimental autoimmune myasthenia gravis [76;77]. Immunization of mice with DNA encoding human tyrosinase-related protein (TRP), but not self TRP, induced both antibody and T-cell responses cross-reactive with self TRP, protecting mice from B16F10 melanoma [78]. When we immunized HER2 Tg mice with heterologous rat neu, HER2 specific T-cell but not antibody response was induced (Fig. 2, right panel). After testing multiple admix vaccines and a number of hybrid constructs, pE2Neu encoding a fusion protein of human HER2 and rat neu was the most effective in overcoming immune tolerance in HER2 Tg mice, where both humoral and cellular immunity were induced [15;16;18;79]. A trial testing this regimen in head and neck cancer patients is open in Padova, Italy (sponsored by INDENA, Italy).

DNA vaccine response in domestic cats

The findings from heterologous and hybrid vaccines indicate a delicate balance between a robust response to heterologous epitopes and reactivity to self-antigen when the vaccine and self DNA share ~90% amino acid (a.a.) sequence identity. This conclusion was put to test in a “First in cats” immunization study [63]. Two heterologous vaccines, *pbearHER2* (95% identity to feline HER2) and pE2Neu (human and rat fusion, 89% identity) were tested in parallel. HER2 sequence homology between species is outlined in Table 1. Cats received DNA electroporation using the NEPA21 electroporator (Nepa Gene, Co.) as in mice. Vigorous antibody and T-cell responses to the heterologous human HER2 antigen were robust, with specific antibody titers exceeding 1,000,000 and T-cell response evident in all immunized cats [63]. These positive results provide the first evidence that DNA electrovaccination is feasible and effective in cats with consistent immunological response.

B-cell tolerance was overcome and antibodies to self-feline-HER2 expressed by 3T3 cells (3T3/*fe*HER2) were induced in all cats whether immunized with heterologous *pbear*HER2 or pE2Neu. This is the first report that immune tolerance in cats is overcome by heterologous HER2 vaccination. T-cell response to self feline HER2 was induced in one of three cats that received either pE2Neu or *pbear*HER2. Several conclusions can be drawn from this “First in cats” vaccination study: (1) DNA electroporation is an effective immunization modality in cats to induce both humoral and cellular immunity, (2) immune tolerance to self HER2 can be overcome by heterologous vaccine, (3) antibody response is more uniform than T-cell response and (4) individual cats have differential T-cell responses.

In another vaccine design, a feline HER2 variant (*pfe*HER2-K) was constructed with a single a.a. substitution Q141K in extracellular subdomain 1, a region often recognized by immune sera [12][63]. Protein modeling analysis (Sybyl-X Suite; Tripos) indicated that Q141K substitution introduced a novel positive charge onto an exposed surface region. Immune sera generated from vaccination with *pfe*HER2-K recognized feline tumor lines K12 and K248 rather than 3T3/*fe*HER2. These findings suggest a focused antibody response to a novel sequence in HER2-K that may mimic a cryptic epitope on tumor cells. Vaccination with plasmid encoding wild type *fe*HER2 did not induce anti-HER2 immunity. Therefore, consistent with the findings in mice, heterologous bear or human/rat fusion HER2 DNA vaccines are more potent than un-modified feline HER2 in overcoming immune tolerance. As expected from the diverse genetic background of outbred cats, HER2 vaccine response is heterogeneous with 30–40% cats producing T-cell response. Importantly, a single point mutation of feline HER2 induced antibodies that recognize unique FMC epitope(s) to show a subtle specificity revealed only in a genuine self-antigen model system.

***In situ* vaccination by non-surgical ablation**

Cancer vaccination initiates an immune interaction to targeted molecules in the tumor. Recognition of additional TAA after DNA vaccination illustrates the potential for further immunization *in situ* [20]. We have tested several strategies to trigger tumor immunity *in situ*. Intratumoral electroporation of D2F2 mammary tumors with cDNA encoding Tetanus toxoid fragment C and IL-12 induced antibody response to Tetanus toxin, T-cell response to endogenous TAA, heavy inflammatory infiltration, partial regression of established tumors and immune memory which protected mice from repeated tumor challenge [80]. These effects are most evident when Tregs are depleted using CD25 mAb, demonstrating a pre-existing, though suppressed immunity to TAA. In a second approach, oncolytic adenovirus (Adv/CD-TK) containing two suicide genes cytosine deaminase (CD) and herpes simplex virus thymidine kinase (TK) [81–84] was injected into the tumor [85]. Immune reactivity to TAA was induced, albeit limited. However, incorporation of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene in the vector amplified the immune response and significantly reduced tumor growth [85;86].

In the 3rd approach, we tested percutaneous cryoablation that utilizes ultra-cold temperatures to ablate cancers of the breast, prostate, kidney, liver, bone, lung, brain and skin [87]. Cryoablation directly induces necrosis by damaging cell membranes and organelles via the formation of ice crystals, and indirectly through osmotic stress and ischemia from

thrombosis of the microvasculature [88]. In addition to tumor debulking, the necrotic tissue becomes a rich reservoir of TAA that are cleared by antigen presenting cells, creating a unique opportunity to prime or boost antitumor immune responses [89]. Using wild type mice bearing TUBO tumors [26], cryoablation alone resulted in systemic immune priming and partial protection upon tumor rechallenge, while little impact was seen in tolerant NeuT mice [26]. Peritumoral injection of the TLR9 agonist CpG following cryoablation significantly eliminated, reduced or delayed local tumor recurrences in WT, NeuT and SCID mice, respectively. Therefore, tumor antigens released by cryoablation induce varying levels of tumor immunity which is enhanced by CpG. Importantly, innate immunity augmented by CpG contributes to tumor eradication as evidenced by the delayed tumor recurrence even in SCID mice. However, long-term recurrence-free survival is dependent on adaptive immunity, which is displayed by the step-wise increase in recurrences observed in WT, tolerant NeuT, and SCID mice. There lies an opportunity for intervention. Cryoablation is a strong test platform because it is an FDA approved procedure that provides consistent tumor ablation. Immune modulating strategies established with cryoablation can be extrapolated to other ablation modalities, such as radiation, irreversible electroporation, or radiofrequency ablation to render *in situ* vaccination.

Monitoring treatment response by molecular imaging

Evaluation of vaccine response, particularly within the tumor microenvironment, can be challenging. The innate and adaptive immune responses occurring within the tumor microenvironment can cause the lesion to appear larger and more metabolically active when imaging with radiolabeled glucose [90;91] by positron emission tomography (PET), potentially confusing whether the tumor is responding to treatment. The ability to distinguish local tumor recurrence from host immune infiltration will be critical for ultimate treatment success. Tools for distinguishing tumor cells and host infiltrates in live animals will be essential. As an example, in pIL-1 β -DsRed Tg mice, increased DsRed intensity, marking IL-1 production, is sustained for 2–3 weeks in cryoablated tumor tissue, displaying sustained inflammation after cryoablation [26].

Amino acid metabolism in the tumor microenvironment can help to further differentiate tumor and immune activity. Tryptophan, an essential amino acid for protein synthesis, is converted to serotonin, kynurenine, and their metabolites to support cell functions (http://www.genome.jp/kegg-bin/show_pathway?hsa00380). An FDA approved PET tracer [^{11}C]-alpha-methyl-tryptophan (^{11}C -AMT), an analogue of tryptophan, is not incorporated into protein, but can instead be converted to alpha-methyl-serotonin or alpha-methyl-kynurenine [92–96]. Tumor cells and other inflammatory cells including Tregs can produce indoleamine-pyrrole 2,3-dioxygenase (IDO), an enzyme that converts tryptophan to kynurenine, which exerts immune suppressive activity. Enhanced AMT retention in well-perfused tissue could indicate IDO activity (Fig. 3); thus, AMT-PET is a potential imaging tool for investigating tryptophan metabolism after local ablation or immune modulation.

Taken together, cancer vaccine research has advanced from pre-clinical to clinical research and the findings warrant intermediate model systems to better predict treatment outcomes in humans. Domestic dogs and cats may provide an advantageous system for resolving issues

of immune tolerance and vaccine response to expedite vaccine development. New insight from feline and canine studies is guiding the design of next generation vaccines. In addition to targeted vaccination, non-surgical ablation of tumor tissue combined with strategic immune modulation can induce *in situ* vaccination to render elevated host immunity. All immune modulating strategies demand an effective monitoring system to guide the treatment plan. Non-invasive imaging technology provides a promising tool to monitor host immunity. Progress in imaging tracer development will further enhance the efficacy of cancer immunotherapy. Through strategic utilization of these new tools, expedited cancer vaccine development can be expected.

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Highlights

- Animal models testing cancer vaccines have advanced to more closely recapitulate human immunity.
- Early mouse models generating spontaneous tumors are generally artificially immunogenic.
- Transgenic mouse models can better mimic natural immune tolerance but still have limitations.
- Domestic cats and dogs parallel human genetic diversity, cancer development and immune tolerance.
- In situ immunization via non-surgical tumor ablation permits recognition of tumor antigen.

Fig. 1.
Evolution of pre-clinical models for cancer vaccine research. Initial breast cancer models began in mice using various transforming agents to induce spontaneous mammary tumors. More advanced mouse models in different strains were created to better recapitulate self-tolerance observed in humans. Outbred dogs and cats can serve as a natural model for tumorigenesis with immunity more representative of humans relative to syngeneic mice.

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Fig. 2.
Development of HER2 DNA vaccines. Immune response to 2nd generation heterologous and hybrid vaccines is summarized (right).

Fig. 3. Metabolic imaging of spontaneous mammary tumors in BALB NeuT mice. (A) PET scan after ^{11}C -AMT administration in a female NeuT mouse with spontaneous mammary tumors (coronal section). AMT activity is visible in mammary glands #1 and 2. AMT activity is also visible in the intestine and bladder from endogenous IDO activity and excretion respectively. (B) Corresponding T2-weighted MRI.

Table 1

HER2 amino acid sequence % identity

	Bear	Cat/Hu hybrid	Human	Pig	Hu/Rat hybrid	Rat	Mouse
Cat	95	95	92	90	89	85	84
Bear		92	91	89	89	84	83
Cat/Hu			97	89	89	85	84
Human				89	93	85	84
Pig					87	83	83
Hu/Rat						91	89
Rat							94