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Genetically Engineered Mouse Models of Distinct Breast Cancer Subtypes

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Abstract

Advances in screening, detection and new therapeutic options have reduced the death rate of women by breast cancer over the last decade. However, despite these advances breast cancer remains the second leading cause of cancer death among women. This is due in part to the complexity of the disease which consists of multiple disease subtypes that are driven by different genetic mechanisms, and likely arise from different cell types of origin. These differences can drive treatment options and outcomes. It is important to choose relevant preclinical model systems to study new therapeutic interventions and tumor biology. This unit describes the characteristics and applications of validated Genetically Engineered Mouse Models (GEMMs) of breast cancer representing the basal-like, luminal and claudin-low human subtypes. The different subtypes have different clinical outcomes in human patients and require different treatment strategies to achieve the best outcomes. These GEMMs can be considered faithful surrogates of their human disease counterparts and represent alternative preclinical tumor models to cell line and patient-derived xenografts in preclinical drug discovery and tumor biology studies.

Introduction

Advances in early screening and detection, as well as new therapeutic options, have reduced the death rate of women by breast cancer over the last decade (SEER, 2012). However, despite these advances breast cancer remains the second leading cause of cancer death among women (SEER, 2012). This is due in part to the complexity of the disease which consists of multiple disease subtypes that are driven by different genetic mechanisms, and likely arise from different cell types of origin (Hoadley et al., 2014). The disease subtype heterogeneity observed in this and other studies directly relates to prognosis and helps to determine what treatment options are available to patients (Lehmann and Pietenpol, 2015; Prat et al., 2015). In particular the subtypes that encompass Triple Negative Breast Cancers (ER-negative, PR-negative, and HER2-negative, i.e. TNBC) are considered some of the worst outcome breast cancers; these TNBC cancers are predominantly of the Basal-like and

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Conflict of Interest

C.M.P is an equity stock holder, and Board of Director Member, of BioClassifier LLC and GeneCentric Diagnostics. C.M.P is also listed an inventor on patent applications on the Breast PAM50, and is a consultant for G1 Therapeutics.

Claudin-Low subtypes (Prat et al., 2013; Prat et al., 2010). These two subtypes represent around 15–20% of all breast cancer cases, with the basal-like subtype showing unique genetic features, many of which it shares with Serous Ovarian Cancers and Lung Squamous cancers (Hoadley et al., 2014). As a consequence of this TNBC heterogeneity, preclinical drug discovery and testing requires that multiple breast cancer models must be used to faithfully recapitulate the spectrum of human disease, even when studying only one clinical disease subtype with TNBC.

Multiple types of *in vivo* models can be utilized to explore human breast tumors including cell-line based xenografts (CDX), patient-derived xenografts (PDX), and Genetically Engineered Mouse Models (GEMMs). (Duncan et al., 2012; He et al., 2015; Howe et al., 2014). While CDX models have long been used in academic research and industry, they have many limitations including the strong selection process for the cell lines to grow first *in vitro*; while it is known that these cell lines maintain their cell type of origin signature, other features are altered including likely differences in growth rates (Ross and Perou, 2001; Ross et al., 2000) and the clonal nature of the resulting CDX lines often lacks the heterogenic therapeutic response observed clinically. CDX models are usually established via subcutaneous inoculation of cells into the flank. This may limit normal tumor-stromal interactions and the use of immune compromised mice also eliminates the host immune system, which is becoming increasing more important with the advent of immune checkpoint inhibitor drugs (Criscitello and Curigliano, 2015; Karn et al., 2015; Yao et al., 2014). Due in part to these limitations CDXs have a poor track record in preclinical testing that can directly translate to similar findings in human patients (Perrin, 2014). PDX models may offer a more robust approach as the tumor cells have never been cultured *in vitro*, but these require extensive infrastructure support to develop and maintain (Gao et al., 2015; Siolas and Hannon, 2013). PDX models must also be grown in immunocompromised mice, which again limits studies using immunotherapies and thus still provides for an altered microenvironment.

The use of GEMMs provides a robust platform to test hypotheses on tumor development, progression, interaction with the microenvironment, and therapeutic response. Multiple GEMMs have been engineered with conditional, often inducible and/or constitutively active mutant alleles (Pletnikov, 2009). As a result, many GEMMs of breast cancer have been developed that reflect some of the diversity of genetic lesions seen in human breast cancer (Herschkowitz et al., 2012; Pfefferle et al., 2013). However, it is often not obvious which human subtype a given GEMM most closely resembles, as common genetic alterations e.g., TP53 loss occur in multiple human breast cancer subtypes. In an attempt to identify those models that most closely reflect human disease subtypes we have gene expression profiled more than 27 different murine GEMM of mammary carcinomas and compared them to multiple human data sets (Herschkowitz et al., 2007; Herschkowitz et al., 2012; Pfefferle et al., 2013). Using this comparative genomics method we have identified murine gene-expression counterparts of the basal-like and claudin-low subtypes and have identified some GEMMs that show tumor features seen in the human luminal subtype (Figure 1).

GEMM Characterization

Based on these genomic results and other parameters (e.g. histology and initiating transgene) three GEMMs were chosen as the basis for multiple studies of therapeutic response (Table 1). Two of these are TNBC models and the third is a model of the Luminal tumor subtype. Representative histology of these models is shown in Figure 2, noting that each model shows dense tumor cell morphology and general adenocarcinoma features, both of which are also seen within high grade human breast tumors. Each GEMM faithfully recapitulates the gene-expression characteristics of their human subtype counterpart and the initiating transgene is also subtype consistent (Pfefferle et al., 2013).

C3-Tag

The FVB-Tg(C3-1-TAg)cJeg (C3-TAg) (Jackson Labs strain 013591) mouse contains a transgenic construct of the Simian Virus 40 T-antigen under the promotion of the rat prostrate binding protein (Maroulakou et al., 1994), which results in males developing prostate tumors around 7 months. Female hemizygous transgenic mice develop mammary gland adenocarcinomas by 21 weeks of age with a penetrance of greater than 90% and with an average latency of palpable mass (approximately 3mm in diameter) of 19 weeks (Figure 3). Untreated animals will progress from initial tumor palpation to a terminal endpoint in an average of 30 days. The consistent high penetrance, short latency and quick progression are positive attributes for work flow, colony management, and therapeutic testing.

Further, the genetics driving tumorigenesis are highly relevant to the human Basal-like disease (i.e. loss of TP53 and RB function). Using a cross-species genomics approach, murine expression profiles were compared to human breast cancers (see Herschkowitz et al. (Herschkowitz et al., 2007) and Pfefferle et al. (Pfefferle et al., 2013) for detailed descriptions). The results of an unsupervised cluster analysis of this combined human and mouse gene expression profiling data set showed gene signatures/modules with obvious shared features that included a proliferation signature and patterns characteristic of each of the human subtypes (see Pfefferle et al. (Pfefferle et al., 2013) for a detailed description. The overwhelming majority (~ 90%) of C3-TAg strain tumors displayed characteristics of human basal-like breast cancer (BLBC) (e.g. high proliferation and high expression of keratins 5, 17, and P-Cadherin) that cluster together as a unique expression group (Figure 1) ; 5–10% of C3-Tag tumors show the claudin-low subtype features and cluster with these tumors. Human BLBCs are RB1-deficient via loss of heterozygosity LOH of the RB1 loci, and BLBCs demonstrate the lowest average expression of RB1 mRNA (Herschkowitz et al., 2007). Studies of many cohorts have also noted that BLBCs exhibit a high frequency of p53 mutation or deletion (Carey et al., 2006) (Troester et al., 2006) (Sorlie et al., 2001). Therefore, it is not surprising that human BLBC is recapitulated by the mouse SV40 T-antigen model C3-TAg, as this viral oncoprotein inactivates both RB1 and TP53. Lastly, we observed a conserved DNA copy number gain in common between human BLBC and the C3-TAg model; both the human and mouse BLBCs showed amplification and high expression of the K-RAS locus (Herschkowitz et al., 2007; Liu et al., 2001), as well as deletion of INPP4B (Herschkowitz et al., 2012), and amplification of nicastrin on human Chromosome 1q21-23 (Silva et al., 2015). Thus, this unbiased credentialing has established

the C3-Tag model as a faithful representative of human BLBC at both the gene expression and DNA somatic alteration levels.

MMTV-Neu

Mouse strain FVB/N-Tg(MMTVneu)202Mul/J (MMTV-Neu) (Jackson Labs strain 002376) expresses the c-NEU oncogene (the rat ortholog of human HER2) driven by the mouse mammary tumor virus (MMTV) promoter (Muller et al., 1996). Penetrance is >90% in females and males are unaffected. The age at which disease presents depends on the individual's breeding status; virgin females develop tumors later while parous females have masses on average by 30 weeks of age. Similar to the C3-Tag, the MMTV-Neu model shows good and consistent colony management characteristics with consistent penetrance, latency times, and similar tumor growth characteristics. The MMTV-Neu allele has the additional benefit of having no deleterious effects if maintained in a homozygous state. Gene expression profiling has shown that this model represents a human luminal subtype, having high expression of XBP1, (a human luminal tumor-defining gene) (Gruvberger et al., 2001). These tumors also expressed tight junction structural component genes, including Occludin, Tight Junction Proteins 2 and 3, and the luminal cell marker K8/18, but they were lacking expression of the estrogen receptor and other estrogen responsive genes (Herschkowitz et al., 2007; Pfefferle et al., 2013). Nonetheless, its clear overall luminal features (Figure 1), consistent latency and easy colony management make it an ideal GEMM for repeated therapeutic testing.

p53 null "T11"

This Orthotopic Syngeneic Transplant (OST) model originates from a mouse with a germline homozygous deletion of p53 (Jerry et al., 2000). Animals deficient for p53 develop spontaneous thymic lymphomas in early life. However donor p53^{-/-} mammary epithelium from young animals can be transplanted into the cleared mammary fat pad of recipient syngeneic (BALB/c) females, and then tumors will develop with a latency of 6–12 months (Jerry et al., 2000). These murine syngeneic transplants can be propagated through serial passage and the individual tumor phenotypes are consistent over time in the large majority of tumors (Herschkowitz et al., 2012). Therefore, OSTs offer the convenience of a cell-line, allowing the user to manipulate workflows as needed by inoculating only those animals required for a given experiment, while also providing a fully immunocompetent model. The particular OST line we have used the most is called "T11", and is available for collaborative research through Baylor College of Medicine. It displays a high take rate of exceeding 99% and a short latency from inoculation to palpable mass of around 12 days. A characteristic of this model is the aggressive nature of tumor progression, which is similar to the human subtype it represents (i.e. claudin-low (Prat et al., 2010). Once a palpable mass is observed the tumor will progress to a terminal burden within 18 days. The tumors develop rapidly and have a propensity to invade the peritoneal cavity.

While many of these p53^{-/-} tumors were generated, 50 were chosen for further characterization that displayed a variety of histological phenotypes and molecular subtypes. Importantly a subset of approximately 10%, were identified as being the murine equivalent to human claudin-low tumors (Herschkowitz et al., 2012). These had low or absent CLDN3

and CDH1 expression and high expression of epithelial–mesenchymal transition (EMT) markers such as SNAIL and TWIST1, similar to the human claudin-low subtype (Prat et al., 2010). The tumor line chosen for subsequent research was Tumor 11 or “T11” (Figure 2).

Translational Application of GEMMs

UNC Lineberger Mouse Phase 1 Unit and “co-clinical” Trials

The Mouse Phase I Unit (MPIU) at the University of North Carolina was founded in 2005 to address the issue of poorly predictive preclinical animal models. Initially relying on private and State of North Carolina funding, the MPIU capitalized on advances in murine genetics as well as changes in the intellectual property landscape (Hanahan et al., 2007) to begin medium-throughput efficacy testing of potential anti-cancer drugs in GEMM. Work in the MPIU relies exclusively on faithful GEMM and OST lines as described above. The MPIU harnesses the capabilities of murine genetics to produce single and/or multi-allelic cancer models featuring autochthonous tumors with physiological tumor-stroma interface in immune competent hosts. Promising anti-cancer compounds are tested alone and in combination in these models, using cohort sizes (10–20 animals per treatment arm) that provide for rigorous statistical results. Tumor response and progression are serially assessed, including novel imaging approaches when needed, using criteria similar to human Response Evaluation Criteria In Solid Tumors (RECIST (Nishino et al., 2010)). As evidence of the consistency of our models, in Figure 3A and B we show the median growth rate of C3-Tag and MMTV-NEU tumors over three consecutive time periods, thus spanning many years and multiple laboratory technicians highlighting the consistency of these models, which is key for performing therapeutic studies over time that compare multiple compounds against each other.

For our therapeutic testing paradigm, we consider an agent effective only if it induces tumor regression (Partial Response (PR) or Complete Response (CR)) rather than mere Tumor Growth Inhibition (TGI). Compound dose, schedule and exposure are monitored through pharmacokinetic (PK) and pharmacodynamic (PD) assessments. Although the MPIU performs only high-level toxicological analysis, testing in the MPIU is otherwise nearly identical to human Phase I/II trials, while also taking advantage of the powerful experimental features of murine tumor models; in essence for each regimen tested, we perform a rolling Phase I/II design where dose finding is performed, and then we immediately move into the randomized Phase II setting. Obviously with animal models, ideal control groups of either no treatment or treatment with the “standard of care” are easily and always performed, as are precise measurement of tumor size changes recorded. Previous groups have identified the value of establishing “co-clinical” trials, where validated *in vivo* models are used as way to expedite drug discovery and identify sensitive patient sub-populations (Chen et al., 2012; Lunardi et al., 2013; Nardella et al., 2011). Below we will outline studies using these three models and methodologies that have informed ongoing clinical questions.

Individual drug and combinatory drug testing

The use of Cyclin Dependent Kinase (CDK) inhibitors may provide benefit for many patients, however there are many kinases associated with the cell cycle pathway, and each may affect a different point in the cell-cycle (Dickson and Schwartz, 2009). Patients given inhibitors against CDK1 and 2 have had adverse events (Sausville et al., 2014), however CDK4/6 inhibitors have been well-tolerated with a select population of patients responding well to these therapies (Turner et al., 2015). For example the CDK 4/6 inhibitor PD0332991 (palbociclib) has shown efficacy in ER+ breast cancer patients receiving an aromatase inhibitor (Mayer, 2015), and has received conditional approval from the FDA for use in the metastatic breast cancer setting (Beaver et al., 2015).

Using GEMMs, we have showed minimal efficacy of palbociclib in *p16^{INK4a}*-null melanoma (Roberts et al., 2012b) and the basal-like breast model C3-TAg, but significant activity in the luminal breast cancer model MMTV-NEU (Roberts et al., 2012a); based on the known genetics of these models, this lack of efficacy in the C3-TAg and *p16^{INK4a}*-null melanoma GEMM is precisely what was predicted (see the Roberts et al. reference (Roberts et al., 2012b) for full experimental results). However, when administered concurrently with carboplatin chemotherapy in the MMTV-Neu model, there was significant inhibition of the anti-tumor properties of carboplatin. In the C3-TAg model there was no similar observation of inhibition of the anti-tumor effects of carboplatin by palbociclib when concurrently administered. Importantly, in the C3-TAg model, the concurrent administration reduced the myelosuppression activity of the carboplatin significantly. Given these and other findings it is likely that CDK4/6 inhibitors will play a role as an anti-cancer therapy, but also as a possible preventative therapy for adverse bone marrow effects as the CDK4/6 inhibitors cause hematopoietic stem cell dormancy, which is immediately reversed upon drug removal (Roberts et al., 2012a).

Combinatorial therapy of novel agents can also be tested reliably in GEMMs to determine broad anti-cancer properties. Combinatorial therapies are particularly challenging to test in humans as there are often unpredictable and toxic side effects of novel combinations; thus the GEMM setting provides a true *in vivo* environment with all host normal tissues present, for which these potential toxic effects can be identified and addressed through changes in doses and schedules. In a recent analysis in Clinical Cancer Research (Usary et al., 2013), we analyzed more than 600 breast cancer GEMM tumors for response to a range of therapeutics focused on MEK, mTOR and PI3CA/mTOR inhibitors. Of note, therapy assessment was performed in more than 300 C3-TAg mice (Figure 5), highlighting a major advantage of GEMMs in that this number of mice is difficult to achieve using PDX models. Of the regimens assessed, the combination of a MEK (AZD6244) and PI3K/mTOR (BEZ235) inhibitor resulted in tumor regression in a significant fraction of animals, and more than doubled overall survival (from 4.5 to 8 weeks). Likewise this combination was equally effective in the Claudin-low T11 model, again doubling overall survival (2 weeks to 4.5 weeks) and providing a significant improvement to Overall Response Rates. We noted, however, that this combination had toxic side effects (weight loss), and it required months of testing of different doses and schedules, to determine the final dose and schedule.

Another advantage of GEMMs is the ability to study, and potentially reverse, acquired resistance. To this end, we first studied the MMTV-NEU models response to lapatinib, where we noted it was exquisitely sensitive (Roberts et al., 2012b). All tumors in this model initially respond to lapatinib, a potent HER2 inhibitor, but a few of these tumors eventually progressed after 100–200 days on therapy. Tumors were harvested from mice with acquired lapatinib resistance and transplanted into mammary fat pads of syngeneic recipients. Mice bearing these tumor-grafts were then re-challenged with lapatinib or other therapies such as AZD6244/BEZ235. Tumors transplanted in this way remained refractory to lapatinib re-challenge, but were highly responsive to the MEK/PI3K/mTOR inhibitor combination (Figure 6). The overall survival of Non-treated mice went from 4 weeks to 16 weeks. Thus through the use of serial transplantation on syngeneic backgrounds, resistant tumors can be selected for, further propagated, and then tested with new agents to identify those that have activity against tumors resistant to common treatments.

Predicting Compound Efficacy

We have also had success using GEMMs and OSTs to develop gene-expression based predictors of response to anticancer drugs in the tumors of human patients (Usary et al., 2013). After testing a number of therapeutic treatments we selected a model and a regimen that showed the strongest heterogeneity of response to a single treatment. The C3-TAg model demonstrated strongly heterogeneous response to treatment with carboplatin and paclitaxel, with one third of tumors showing regression and two thirds tumor growth (Usary et al., 2013). Microarray analysis revealed a set of genes that were differentially regulated in the untreated mice relative to the treated cohort (Figure 7). The murine genes were converted to their human orthologs which were then used to cluster a human breast cancer microarray set. This mouse to human “filtering” process was required because homogenous gene lists derived from murine tumors or cell line sources will typically fragment into smaller gene sets when analyzed on human tumors. We next selected and tested individual dendrogram node gene sets that contained highly homogeneously expressed gene sets for predictive value in a set of patients that had received taxane and anthracycline containing neoadjuvant chemotherapy. One of these gene sets predicted a pathological complete response (pCR) across all tumor types and even within the Basal-like subtype and the triple-negative clinical grouping. Multivariate analysis showed that the originally murine derived gene signature provided predictive information for pCR beyond the commonly used clinical variables and breast tumor subtype. These results showed that murine-derived gene signatures can predict response in human patients after accounting for other clinical variables and suggest that these murine GEMM models may be useful in identifying new biomarkers for human breast cancer patients.

Summary

Drug discovery in oncology has historically been predicated on *in vitro* cell line models, and/or cell lines grown in immune compromised mice. However, there are a number of limitations to their use including limited genetic complexity, artificial tumor-host stroma interaction and lack of a host immune response. GEMMs provide an alternative platform that addresses these shortcomings, and in conjunction with CDXs and PDXs, provides

strong preclinical validation to move drug candidates into the clinic and inform which subsets of patients may benefit from targeted therapies (i.e. which disease subtype is sensitive to a given regimen). Previous work in the UNC MPIU has shown that our genomically selected GEMMs faithfully recapitulate specific human disease counterparts that represent important subtypes of breast cancer. Furthermore the phenotype (tumor growth, latency and breeding) of these GEMMs allow for easy colony management and reduces the infrastructure demands that a similarly sized PDX colony would require. Importantly as the host immune system becomes increasingly critical to therapeutic intervention of human cancer, GEMMs will provide a valuable platform for drug testing.

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Literature Cited

- Beaver JA, Amiri-Kordestani L, Charlab R, Chen W, Palmby T, Tilley A, Zirkelbach JF, Yu J, Liu Q, Zhao L, Crich J, Chen XH, Hughes M, Bloomquist E, Tang S, Sridhara R, Kluetz PG, Kim G, Ibrahim A, Pazdur R, Cortazar P. FDA Approval: Palbociclib for the Treatment of Postmenopausal Patients with Estrogen Receptor-Positive, HER2-Negative Metastatic Breast Cancer. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2015; 21:4760–4766. [PubMed: 26324739]
- Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA*. 2006; 295:2492–2502. [PubMed: 16757721]
- Chen Z, Cheng K, Walton Z, Wang Y, Ebi H, Shimamura T, Liu Y, Tupper T, Ouyang J, Li J, Gao P, Woo MS, Xu C, Yanagita M, Altabef A, Wang S, Lee C, Nakada Y, Pena CG, Sun Y, Franchetti Y, Yao C, Saur A, Cameron MD, Nishino M, Hayes DN, Wilkerson MD, Roberts PJ, Lee CB, Bardeesy N, Butaney M, Chirieac LR, Costa DB, Jackman D, Sharpless NE, Castrillon DH, Demetri GD, Janne PA, Pandolfi PP, Cantley LC, Kung AL, Engelman JA, Wong KK. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. *Nature*. 2012; 483:613–617. [PubMed: 22425996]
- Criscitiello C, Curigliano G. Immunotherapy of Breast Cancer. *Progress in tumor research*. 2015; 42:30–43. [PubMed: 26377084]
- Dickson MA, Schwartz GK. Development of cell-cycle inhibitors for cancer therapy. *Curr Oncol*. 2009; 16:36–43. [PubMed: 19370178]
- Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, Johnson NL, Granger DA, Jordan NV, Darr DB, Usary J, Kuan PF, Smalley DM, Major B, He X, Hoadley KA, Zhou B, Sharpless NE, Perou CM, Kim WY, Gomez SM, Chen X, Jin J, Frye SV, Earp HS, Graves LM, Johnson GL. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell*. 2012; 149:307–321. [PubMed: 22500798]
- Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, Balbin OA, Barbe S, Cai H, Casey F, Chatterjee S, Chiang DY, Chuai S, Cogan SM, Collins SD, Dammasa E, Ebel N, Embry M, Green J, Kauffmann A, Kowal C, Leary RJ, Lehar J, Liang Y, Loo A, Lorenzana E, Robert McDonald E 3rd, McLaughlin ME, Merkin J, Meyer R, Naylor TL, Patawaran M, Reddy A, Roelli C, Ruddy DA, Salangsang F, Santacrose F, Singh AP, Tang Y, Tinetto W, Tobler S, Velazquez R, Venkatesan K, Von Arx F, Wang HQ, Wang Z, Wiesmann M, Wyss D, Xu F, Bitter H, Atadja P, Lees E, Hofmann F, Li E, Keen N, Cozens R, Jensen MR, Pryer

- NK, Williams JA, Sellers WR. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature medicine*. 2015; 21:1318–1325.
- Gruvberger S, Ringner M, Chen Y, Panavally S, Saal LH, Borg A, Ferno M, Peterson C, Meltzer PS. Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. *Cancer research*. 2001; 61:5979–5984. [PubMed: 11507038]
- Hanahan D, Wagner EF, Palmiter RD. The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. *Genes Dev*. 2007; 21:2258–2270. [PubMed: 17875663]
- He Z, Schulz A, Wan X, Seitz J, Bludau H, Alakhova DY, Darr DB, Perou CM, Jordan R, Ojima I, Kabanov AV, Luxenhofer R. Poly(2-oxazoline) based micelles with high capacity for 3rd generation taxoids: preparation, in vitro and in vivo evaluation. *Journal of controlled release: official journal of the Controlled Release Society*. 2015; 208:67–75. [PubMed: 25725361]
- Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, Rasmussen KE, Jones LP, Assefnia S, Chandrasekharan S, Backlund MG, Yin Y, Khrantsov AI, Bastein R, Quackenbush J, Glazer RI, Brown PH, Green JE, Kopelovich L, Furth PA, Palazzo JP, Olopade OI, Bernard PS, Churchill GA, Van Dyke T, Perou CM. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biol*. 2007; 8:R76. [PubMed: 17493263]
- Herschkowitz JI, Zhao W, Zhang M, Usary J, Murrow G, Edwards D, Knezevic J, Greene SB, Darr D, Troester MA, Hilsenbeck SG, Medina D, Perou CM, Rosen JM. Comparative oncogenomics identifies breast tumors enriched in functional tumor-initiating cells. *Proc Natl Acad Sci U S A*. 2012; 109:2778–2783. [PubMed: 21633010]
- Hoadley KA, Yau C, Wolf DM, Cherniack AD, Tamborero D, Ng S, Leiserson MD, Niu B, McLellan MD, Uzunangelov V, Zhang J, Kandoth C, Akbani R, Shen H, Omberg L, Chu A, Margolin AA, Van't Veer LJ, Lopez-Bigas N, Laird PW, Raphael BJ, Ding L, Robertson AG, Byers LA, Mills GB, Weinstein JN, Van Waes C, Chen Z, Collisson EA, Cancer Genome Atlas Research, N. Benz CC, Perou CM, Stuart JM. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell*. 2014; 158:929–944. [PubMed: 25109877]
- Howe MK, Bodoor K, Carlson DA, Hughes PF, Alwarawrah Y, Loiselle DR, Jaeger AM, Darr DB, Jordan JL, Hunter LM, Molzberger ET, Gobillot TA, Thiele DJ, Brodsky JL, Spector NL, Haystead TA. Identification of an allosteric small-molecule inhibitor selective for the inducible form of heat shock protein 70. *Chemistry & biology*. 2014; 21:1648–1659. [PubMed: 25500222]
- Jerry DJ, Kittrell FS, Kuperwasser C, Laucirica R, Dickinson ES, Bonilla PJ, Butel JS, Medina D. A mammary-specific model demonstrates the role of the p53 tumor suppressor gene in tumor development. *Oncogene*. 2000; 19:1052–1058. [PubMed: 10713689]
- Karn T, Pusztai L, Rody A, Holtrich U, Becker S. The Influence of Host Factors on the Prognosis of Breast Cancer: Stroma and Immune Cell Components as Cancer Biomarkers. *Current cancer drug targets*. 2015; 15:652–664. [PubMed: 26452382]
- Lehmann BD, Pietenpol JA. Clinical implications of molecular heterogeneity in triple negative breast cancer. *Breast (Edinburgh, Scotland)*. 2015
- Liu ML, Shibata MA, Von Lintig FC, Wang W, Cassenaer S, Boss GR, Green JE. Haploid loss of K-ras delays mammary tumor progression in C3 (1)/SV40 Tag transgenic mice. *Oncogene*. 2001; 20:2044–2049. [PubMed: 11360188]
- Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, Wang G, Mazzucchelli R, Bianconi M, Stack EC, Lis R, Patnaik A, Cantley LC, Bubley G, Cordon-Cardo C, Gerald WL, Montironi R, Signoretti S, Loda M, Nardella C, Pandolfi PP. A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. *Nat Genet*. 2013; 45:747–755. [PubMed: 23727860]
- Maroulakou IG, Anver M, Garrett L, Green JE. Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40 large tumor antigen fusion gene. *Proc Natl Acad Sci U S A*. 1994; 91:11236–11240. [PubMed: 7972041]
- Mayer EL. Targeting breast cancer with CDK inhibitors. *Curr Oncol Rep*. 2015; 17:443. [PubMed: 25716100]
- Muller WJ, Arteaga CL, Muthuswamy SK, Siegel PM, Webster MA, Cardiff RD, Meise KS, Li F, Halter SA, Coffey RJ. Synergistic interaction of the Neu proto-oncogene product and transforming

growth factor alpha in the mammary epithelium of transgenic mice. *Mol Cell Biol*. 1996; 16:5726–5736. [PubMed: 8816486]

Nardella C, Lunardi A, Patnaik A, Cantley LC, Pandolfi PP. The APL paradigm and the “co-clinical trial” project. *Cancer Discov*. 2011; 1:108–116. [PubMed: 22116793]

Nishino M, Jagannathan JP, Ramaiya NH, Van den Abbeele AD. Revised RECIST guideline version 1.1: What oncologists want to know and what radiologists need to know. *AJR Am J Roentgenol*. 2010; 195:281–289. [PubMed: 20651182]

Perrin S. Preclinical research: Make mouse studies work. *Nature*. 2014; 507:423–425. [PubMed: 24678540]

Pfefferle AD, Herschkowitz JI, Usary J, Harrell JC, Spike BT, Adams JR, Torres-Arzayus MI, Brown M, Egan SE, Wahl GM, Rosen JM, Perou CM. Transcriptomic classification of genetically engineered mouse models of breast cancer identifies human subtype counterparts. *Genome Biol*. 2013; 14:R125. [PubMed: 24220145]

Pletnikov MV. Inducible and conditional transgenic mouse models of schizophrenia. *Prog Brain Res*. 2009; 179:35–47. [PubMed: 20302816]

Prat A, Adamo B, Cheang MC, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist*. 2013; 18:123–133. [PubMed: 23404817]

Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast cancer research: BCR*. 2010; 12:R68. [PubMed: 20813035]

Prat A, Pineda E, Adamo B, Galvan P, Fernandez A, Gaba L, Diez M, Viladot M, Arance A, Munoz M. Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast (Edinburgh, Scotland)*. 2015

Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, Zamboni WC, Wong KK, Perou CM, Sharpless NE. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst*. 2012a; 104:476–487. [PubMed: 22302033]

Roberts PJ, Usary JE, Darr DB, Dillon PM, Pfefferle AD, Whittle MC, Duncan JS, Johnson SM, Combest AJ, Jin J, Zamboni WC, Johnson GL, Perou CM, Sharpless NE. Combined PI3K/mTOR and MEK inhibition provides broad antitumor activity in faithful murine cancer models. *Clin Cancer Res*. 2012b; 18:5290–5303. [PubMed: 22872574]

Ross DT, Perou CM. A comparison of gene expression signatures from breast tumors and breast tissue derived cell lines. *Disease markers*. 2001; 17:99–109. [PubMed: 11673656]

Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M, Pergamenschikov A, Lee JC, Lashkari D, Shalon D, Myers TG, Weinstein JN, Botstein D, Brown PO. Systematic variation in gene expression patterns in human cancer cell lines. *Nature genetics*. 2000; 24:227–235. [PubMed: 10700174]

Sausville E, Lorusso P, Carducci M, Carter J, Quinn MF, Malburg L, Azad N, Cosgrove D, Knight R, Barker P, Zabludoff S, Agbo F, Oakes P, Senderowicz A. Phase I dose-escalation study of AZD7762, a checkpoint kinase inhibitor, in combination with gemcitabine in US patients with advanced solid tumors. *Cancer Chemother Pharmacol*. 2014; 73:539–549. [PubMed: 24448638]

SEER. 2012.

Silva GO, He X, Parker JS, Gatz ML, Carey LA, Hou JP, Moulder SL, Marcom PK, Ma J, Rosen JM, Perou CM. Cross-species DNA copy number analyses identifies multiple 1q21–q23 subtype-specific driver genes for breast cancer. *Breast cancer research and treatment*. 2015

Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. *Cancer research*. 2013; 73:5315–5319. [PubMed: 23733750]

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001; 98:10869–10874. [PubMed: 11553815]

Troester MA, Herschkowitz JI, Oh DS, He X, Hoadley KA, Barbier CS, Perou CM. Gene expression patterns associated with p53 status in breast cancer. *BMC Cancer*. 2006; 6:276. [PubMed: 17150101]

- Turner NC, Ro J, Andre F, Loi S, Verma S, Iwata H, Harbeck N, Loibl S, Huang Bartlett C, Zhang K, Giorgetti C, Randolph S, Koehler M, Cristofanilli M. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. *The New England journal of medicine*. 2015
- Usary J, Zhao W, Darr D, Roberts PJ, Liu M, Balletta L, Karginova O, Jordan J, Combest A, Bridges A, Prat A, Cheang MC, Herschkowitz JI, Rosen JM, Zamboni W, Sharpless NE, Perou CM. Predicting drug responsiveness in human cancers using genetically engineered mice. *Clin Cancer Res*. 2013; 19:4889–4899. [PubMed: 23780888]
- Yao J, Caballero OL, Yung WK, Weinstein JN, Riggins GJ, Strausberg RL, Zhao Q. Tumor subtype-specific cancer-testis antigens as potential biomarkers and immunotherapeutic targets for cancers. *Cancer immunology research*. 2014; 2:371–379. [PubMed: 24764584]

Figure 1. Hierarchical clustering of Genetically Engineered Mouse Models

Unsupervised clustering of mRNA expression levels reveals the stability of the GEMM and Orthotopic Syngeneic Transplant (OST) tumor murine models across time, and across multiple microarray platforms. Each column indicates an individual microarray experiment of a single tumor, and each row represents an mRNA transcript with red indicating the highest, black intermediate, and green the lowest relative levels of expression. The Agilent arrays were done over a span of twelve years using 22,000, 44,000 and 180,000 feature array platforms. Microarrays done on the three models described here are seen grouped together on the same dendrogram branches (identified above), thus showing the robustness of the genomic analysis, and of the biology of each GEMM model in question (Neu= luminal GEMM, C3-Tag=basal-like, T11=claudin-low subtype).

Figure 2. Murine tumor immunohistochemistry

Representative histology of H&E stained tissue sections from C3-TAg (A and D), MMTV-Neu (B and E), and T11 (C and F) murine tumors.

Figure 3. Latency and progression of three Breast GEM Models

A) Latency is the time from birth, or inoculation in the case of the T11 model, to a palpable mass. T11 had the earliest latency with a mean of 12.6 days (range of 9–18). C3-Tag developed a mass on average at 19 weeks (13–22). The MMTV-Neu, from a parous state, developed masses at 30 weeks (16–52). B) Progression is the time from palpable mass to terminal burden in days. The T11 was the most aggressive with a progression of 15 days. While the C3-Tag and MMTV-Neu grew more slowly with mean progressions of 30 and 37 days.

Figure 4. Model Fidelity

The percent increase in tumor size from initial time of detection to after fourteen days for the A) C3-TAg and B) MMTV-Neu models. Data are compared between three time periods (2010–2011, 2011–2013, and 2013–2015), which spans multiple technicians, and thus highlights the consistency of these models over time.

Figure 5. Treatment Response. Fourteen-day tumor response of Triple Negative Breast GEM Models

Dotted lines show Stable Disease cutoff points. Of 155 C3-TAGs treated, AZD6244/BEZ235 achieved an Objective Response (Stable + Regressive Disease) in 17 of 20 animals, which was the highest percentage of any treatment assessed. B) Of 99 T11 mice treated 7 of 13 achieved an Objective Response when treated with AZD6244/BEZ235.

Figure 6. Development and re-challenge of MMTV-Neu lapatinib resistant tumors

A) Treatment of an MMTV-Neu mouse with lapatinib (220mpk) resulted in tumors that shrank to a size nearly undetectable. After extended treatment, one of the tumors developed a resistant phenotype and rapidly regrew. B) When the resistant tumor was serially transplanted into multiple syngenic background (FVB) mice the tumors remained resistant to lapatinib but were susceptible to other tyrosine kinase inhibitors.

Figure 7. Predicting drug efficacy in human patients

(A) Differentially expressed murine genes were identified and (B) were converted their human orthologs. (C) These gene sets were used to cluster microarrays of human patient tumors and (D) gene signatures were derived from sets of the genes that were most homologously expressed. (E) The resulting gene signatures were tested against patient response data to find the ones that provided predictive power.

Table 1

Mouse Models.
Presented GEM Models of Breast Cancer

GEM	Name	References	Median Latency	Source	Subtype
FVB-Tg(C3-1-TAg)	C3-TAg	PubMed: 7972041	18.3wks	Jackson Labs, strain 013591	basal
FVB/N-Tg(MMTVneu)	MMTV-Neu	PubMed: 1359541, PubMed: 8816486	variable, parous females 18wks	Jackson Labs, strain 002376	luminal
OST <i>p53null/null</i>	T11	PMID: 21633010, PMID: 10713689	~ 10 daysfrom inoculation	Baylor College of Medicine	claudin-low