Editorial Commentary



# Of mice and men: pre-clinical models to identify therapy responsive patient subgroups

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Ovarian cancer is the eighth leading cause of cancer related death among women, accounting for more than 150,000 deaths annually worldwide (1,2). High-grade serous ovarian cancer (HGSOC) is the most malignant form of ovarian cancer and accounts for approximately 70% of ovarian cancer diagnosis. Studies on cancer initiation, growth and metastasis have typically focused on genetic derangements in neoplastic cells; however, tumor growth cannot be exclusively explained by aberrations in cancer cells. Thus, it is of great interest to have a comprehensive understanding of how the tumor microenvironment (TME) promotes the neoplastic niche, and ultimately how to target the TME (including tumor stroma, extracellular matrix, and immune cells) to reduce disease recurrence and drug resistance.

Historically, preclinical models focus on the genetic characteristics of the epithelial cells and have lacked in maintaining relevant TME components. In a recent study by Maniati *et al.* (3), the authors focused on characterizing the epithelial compartment and the TME of orthotropic syngeneic mouse tumor models to determine their analogy to patient tumors and to what extent these models can be utilized in preclinical studies that test TME targeting therapeutics. Six metastatic omental models of HGSOC were characterized. Two models, 30200 and 60577, were developed from genetically engineered mouse models (GEMMs) which had been engineered for *Trp53*$^{-/-}$, *Brca*$^{-/-}$ and inactivation of the tumor suppressor function of *Rb*. Four additional models were developed from GEMMs with fallopian tube specific inducible inactivation of *Brca2*, *Trp53*, and *Pten* (models HGS1-4). RNA sequencing (RNA-seq) analysis revealed nearly 1,300 differentially expressed genes [false discovery rate (FDR) <0.05] in the murine tumors compared to normal omental tissue. As expected, much of the tumor proliferation and survival pathways were significantly enriched (P<0.001).

Copy number variation (CNV) frequently contributes to the alteration of oncogenic drivers or the deletion of tumor suppressors. HGSOC tumors have relatively more CNVs than many other tumor types, where patients have a medium fraction of 46% of their genome altered (4), compared to approximately 5–10% in various other cancer types (5). Typical preclinical models use immune-deficient HGSOC xenograft models with established cell lines. Much of the common cell lines used for *in vivo* modeling lack the CNV profiles that are commonly found in patient tumors further confirming a loss of genetic fidelity in historically used xenograft models. A study from Domcke *et al.* evaluating the genetic profile of 47 ovarian cell lines revealed profound differences in copy-number changes, mutations and mRNA expression of 12 of the most readily used ovarian cancer cells compared to patient tumor profiles (4). Due to a lack of genetic fidelity of cell lines, researchers have put much effort into developing models that better recapitulates the genetic profile of HGSOC, such as patient

derived xenografts (PDX). The copy number of PDX lines are highly correlated (Pearsons r ≥0.8) with their patient-matched tumor sample, suggesting that the PDX lines are more suitable models of HGSOC than most established ovarian cell lines (6). In general PDX models recapitulate the biology of the original patient tumor and mimic drug response to that of the patients better than historic cell line models for HGSOC (6-8).

To determine the genomic fidelity of the new GEMM murine models, Maniati and colleagues performed RNAseq analysis on tumor samples from the GEMM murine models, primary patient tumors, and metastatic lesions. Subsequent interrogation using a Gaussim graphical model determined a significant correlation in the expression of hallmark signaling pathways (e.g., p53, DNA damage, and mTOR signaling) that were enriched concordantly in all samples. A comparative genomic hybridization copy number analysis further identified gain and loss of genes, including some of the 20 most significant recurrent CNVs, including amplification in *Myc*, *PI3K*, *mTOR* and *NOTCH* signaling pathways, indicating that that the GEMM models recapitulates the genomics of patient tumors.

Recent sequencing efforts to unveil the genomic landscapes of the TME has determined significant correlations between the ECM and related components, or the matrisome, patient prognosis, and immune response (9,10). The Maniati study found that in the murine GEMM-derived xenograft models, the TME-related pathways (e.g., regulation of cytokine secretions, response to mechanical stimulus, transforming growth factor b (TGF-b) stimulation, cellular response to hypoxia, ECM-receptor interactions, wound healing, immune and inflammatory responses, and angiogenesis) were also significantly enriched compared to normal omental tissue (3). These results demonstrate that key pathways associated with matrisome and immune response are upregulated in the GEMM models, and that these changes are similar to pathway activation in patient tumors.

In an earlier study, the authors conducted multi-layered TME profiling of evolving omental metastases of human HGSOC samples (9). They defined a 22-gene matrisome signature that can predict the extent of disease, tissue remodeling, and tissue stiffness, and found that all 22 of these orthologous genes from the Matrix Index were upregulated, albeit at variable levels of expression, in all GEMM tumors. This suggests that human and murine tumors share common differential expression of the TME. In contrast, Lim *et al.* developed a tumor matrisome index (TMI; also known as the EMC-related prognostic and predictive indicator, or EPPI) comprised of 29-matrisome-genes that not only was predictive in patient prognosis but also useful in prioritizing TME-targeting therapeutic approaches in early-stage non-small cell lung cancer (10). Until recently, it has been unknown to what extent the matrisome pattern is conserved in progressive tumors across diverse cancer types or if the prognostic value of the TMI could be applied to other tumor types. Lim *et al.* performed a follow up study to analyze the tumor TMI, in over 30,000 patient-derived samples across 11 major cancer types (11). Combined quantitative analyses of genomics and proteomics revealed that TMI is closely associated with mutational load, tumor pathology, and predicts survival across different malignancies. $TMI_{high}$ was an unfavorable prognostic factor for overall survival in colon, liver, renal, and breast cancers, whereas it appeared to confer a favorable prognosis in ovarian and gastric cancers. These results contradict the Maniati study which positively correlates the Matrix Index to patients with shorter overall survival (3). In the Maniati study, upregulation of murine and human samples, *COL11A*, a gene that encodes for collagen synthesis and is associated with poor prognosis in epithelial cancer (12), was one of the most consistently upregulated genes in all models analyzed (3). Interestingly, only two genes, *COL11A1* and *COL6A6*, overlapped between the two different matrix indexes, likely accounting for the profound differences in prognostic values of the two matrisome index studies. Three additional genes, *RUNX2*, *VDR*, and *FOLR1*, which were overexpressed in the murine models, have direct relation to the human Matrix Index. *RUNX2* is a transcription factor that is shared by most genes determined to be associated with the human Matrix Index (3). *FOLR1* is a gene commonly over expressed in ovarian cancer and a target for various approaches of immunotherapy such as targeting by CAR-T cells and cancer vaccines (13-15).

It is well established that cancer associated fibroblasts contribute to ovarian cancer growth and metastasis, and thus has become an increasingly attractive target for anti-cancer therapies (16,17). In PDX models, tumor-associated stroma are almost completely replaced by murine-derived ECM and fibroblasts after 3–5 passages. This new murine stroma is likely to cause drastic changes in paracrine regulation of the PDX tumors as well as in physical properties such as interstitial fluid pressure, which may disrupt drug penetrance (18). The newly characterized GEMM-derived mouse models had a similar genomic pattern of fibroblast specific genes, compared to the primary patient tumor.

Moreover, the amount and pattern of collagen deposition and accumulation is consistent between models and species. Prior reports suggest that there is a strong correlation between the density of fibroblastic cells that are positive for α-smooth muscle actin (α-SMA) and the tissue modulus of the samples, which is further correlated to disease score (9). Using the mechanical indentation method applied to probe the mechanical properties of the PDX and patient tumor tissue (19), Maniati *et al.*, found that mouse tissues were 1–2 orders of magnitude higher than the normal human or mouse omentum, which is the same range as heavily diseased human tissue.

Cell line and PDX models lack immune cells, making it difficult to study immune modulating therapies. To overcome this challenge, researchers have focused on developing *humanized* PDX models. Generally, these models are reconstituted with healthy donor PBMCs or human CD34+ cells, neither of which possess autologous anti-tumor activity and are frequently allogeneic donors which often result in graft-versus- host disease (20). To overcome these deficiencies, we recently developed an orthotopic PDX model of HGSOC that is transferred with autologous tumor infiltrating lymphocytes (TILs). Our model showed HLA-mediated anti-tumor efficacy that was enhanced by immune checkpoint inhibitors, suggesting a novel autologous tumor/TIL model with anti-tumor reactivity (21). However, even with this model, there remains some inherent limitations as it generally lacks the human innate immune cells, stroma and tumor vasculature that may serve as natural endogenous barriers to of effective immunotherapies.

To identify the immune cell subtypes and phenotypes associated with orthotopic murine models, Maniati *et al.* used CIBERSORT (22) to interrogate the transcriptional composition of tumors. All human and murine tumors that were interrogated had high levels of genes encoding for monocytes, macrophages, B cells, CD4+ and CD8+ T cell, as well as variable expression of genes associated with resting dendritic cells, neutrophils and natural killer (NK) cells. To confirm these results, murine and human tumors were analyzed by flow cytometry and some differences between the two species were identified. Mice tumors had a detectible population of macrophages, monocytes, B cells, granulocytes, CD4+ T cells, and a small population of CD8+ T cells and CD11c- dendritic cells. There was a significantly reduced number of T-cells in the murine tumors compared to the human tumors ($P<0.001$), whereas pan-macrophage marker F4/80 cell density (as determined by IHC) was similar to all mouse models with the exception of model 60577. As the authors state, the higher overall quantity of TAMs and lower numbers of CD3+ T cells (with a higher proportion of CD4+ T cells) in the mouse versus human tumors is likely the most substantial differences between the two models, that could impact a response to immunotherapies.

Overall, the tumor models that were developed in this study (3) and by others (6,7,21) show that the genetic heterogeneity is retained in patient tumors and PDX models. Although, hierarchal clustering shows genetic differences between cell line-derived GEMM models and patient tumors (3). As the distinct heterogeneity of the GEMM tumors are retained overtime it could be possible to exploit the genetics of the models. Model 60577 tumors have an increase in cell-cycle pathway activation compared to the other 5 models characterized here. Hence, it was hypothesized that this model would be more responsive to carboplatin therapy. Survival of 60577-bearing mice was increased to 5-fold by platinum treatment compared to <2-fold for HGS2-bearing mice. Moreover, GEMM models correctly predicted the susceptibility to anti-cancer drugs in 70% of an analyzed patient consortium, when correlating the genomic profiles of the models and patient tumors. An even more exciting study was performed to determine if you can exploit vulnerabilities of the models and modulate the TME. 30200 tumors had higher expression of the interleukin-6 (IL-6) pathway. After 5–7 weeks of anti-murine IL-6 antibodies, 30,200 tumor-bearing mice had overall increased survival and the tumors had less tumor-associated macrophages (TAMs), less expression of tumor-promoting TAM marker CD206, and increased intra-tumor CD3+ T cells. These results did not translate to the models with the lowest IL-6 pathway activation. As there are some key differences in the innate and adaptive immune response in these GEMM models compared to endogenous patient response improvements could be made to testing immune modulating therapies. Ideally, a patient-derived model reconstituted with an autologous immune component that has complete and fully functioning innate and adaptive components would further advance this research. Unlike the GEMM models a patient-derived model would allow for a (I) more accurate representation of a clinical immune response and (II) testing clinically developed therapeutics that lack murine cross reactivity.

The findings by Maniati *et al.* are important as they provide a proof-of-concept that syngeneic mouse tumor models have similar biomechanical, cellular and molecular

 

features of human disease, which are characterized by mRNA expression profiles, innate and adaptive immune responses, tissue modulus, and matrisome components, highlighting the utility of these as representative model systems for the study and treatment of human disease.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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