Cell lines remain the ultimate tool for cell biologists and scientists in laboratories all over the world. For decades, the use of cell lines has led to tremendous discoveries in basic science and to fundamental progress in translational medicine towards novel drug developments. In cancer research, huge effort has been put forward to establish cancer cell lines isolated from patients and toward making them available to the research community. Such work has created a unique resource for cancer researchers and has shaped the fundamentals of cancer research.

Until now, it has generally been accepted that the genome of cancer cell lines remains genetically stable over time even though conflicting results in drug responses have been reported. In a recent study published in Nature, Golub and colleagues from the Broad Institute and Dana-Farber Cancer Center (MA, USA) challenged this dogma and revealed substantial genetic changes with functional consequences in over 100 human cell lines. For example, different sub-lines of the parental estrogen-receptor (ER)-positive breast cancer cell line MCF7, which is widely used in cancer research, were collected and their genetic profiles were analyzed. They showed that these sub-lines exhibited various genetic changes such as differential loss or gain of commonly altered genes in breast cancer. PTEN, a major oncogenic driver, was deleted in 17 out of 27 sub-lines. Overall 654 genes were differentially expressed when comparing just two of the sub-lines. Such differences consequently led to fundamental changes in important biological pathways.

Sub-lines with inactivating PTEN showed reduced PTEN signaling with increase in the mTOR gene signature. One can imagine that such changes similarly led to significant variations in drug responses and resistance. By testing 321 different drugs, the group discovered that around 75% of the compounds showed efficacy in some sub-lines but were completely ineffective in others. A clear correlation was observed between drug efficacy and the genetic features of the sub-line as well as the expression level of the genes targeted by the drug. The data suggest that such genetic drift is due to the selection of a pre-existing sub-clone most likely determined by the culture conditions and passing. Interestingly, one of the sub-lines which was cultured and passaged in an in vivo mouse system showed the most distinct separation to all others.

Overall, these results are concerning, given that cell culture is a fundamental aspect of basic cancer research studies. This concern is particularly relevant in the era of precision and personalized medicine where candidate drugs target a specific genetic profile. The variations revealed in the study by Golub and colleagues will affect not only the reproducibility of results but more importantly also the predictability and translatability of the results for clinical implication. These observations clearly indicate that more rigid practice standards for the use of cell lines in the laboratory need to be established and followed.

Functional and phenotypic artifacts induced by cell lines grown in a dish have been recognized for decades. It is well established that the flat, stiff surfaces of culture dishes as well as stable pH and high concentrations of nutrients and oxygen are far off from the natural environment within which cancer cells usually reside. To circumvent these issues, patient-derived xenograft (PDX) models have been generated and are becoming the gold standard for cancer drug testing. In PDX models, rather than creating xenografts using cancer cell lines, tumor samples freshly isolated from a patient are directly implanted and passaged in living mice. An example of one of the advantages of PDX models is that they can, in some clinical contexts, help predict the patient’s outcome and thus guide treatment options to increase clinical benefit. Overall, PDX models are considered to better reflect and represent the cellular and molecular characteristics of human cancers. However, one major drawback of these models is that the mice have an impaired immune system that is species-mismatched with the exogenous human tissue. That makes it impossible to use PDX models for the development and testing of immunotherapies. To address this issue, Keck and colleagues at the Jackson Laboratory (ME, USA) recently published a mouse model in FASEB Journal that incorporates aspects of the human immune system. Challenges remain; however, as this model probably will also face common issues already seen in PDX models: the transplanted human tumor piece contains human vascular and connective tissue which over time are gradually replaced by the mice’s own tissue. One can imagine that these tissues are vastly different in a mouse compared to a human and already small differences in size and density can affect the penetration of various drugs tested. Moreover, in an earlier study published in 2017 in Nature Genetics Golub and colleagues demonstrated that genetic drift as seen in cell lines can similarly occur in PDX samples. By comparing the genetic profile of 1,110 PDX samples across 24 cancer types, they found that 60% of all PDX models acquired at least one chromosomal aberration after only one single in vivo passage. After four passages, the percentage increased to 88%. Such changes were not limited to copy number alterations, but also affected mutations in major oncogenic pathways. Similar results were also obtained testing cell line-derived xenografts. Most concerning is the finding that tumor evolution was significantly different between the PDX models and human cancers indicating a murine-specific selection pressures. Such selection mechanism induced by the murine host were associated with changes in sensitivity and responses to targeted drugs, challenging the potential and usability of such models for drug development.

No model system is perfect, especially when it comes to complex diseases such as cancer. It is obvious that preclinical studies and outcomes may not always reflect what happens in cancer patients. Given this backdrop, it is not surprising why so many drugs developed based on mouse models ultimately failed when tested in the clinic. As a research community, more efforts are needed to provide better model.
systems and computational tools that are more predictive for drug responses and efficacy in humans.

*EBioMedicine* encourages the use of patient-derived primary cells and materials for the purpose of understanding disease mechanisms, identifying therapeutic targets and testing drug candidates. We strongly encourage regular genetic testing and limitation of passage numbers, as well as clear documentation of cell culture conditions used in the study. Furthermore, we promote sharing of de-identified clinical data and tissue material by making such information and databases available and more accessible to the research community. Ideally, a comprehensive characterization of cell lines and model systems should be required for preclinical studies in order to ensure reproducibility. At *EBioMedicine* we ask that authors provide a statement outlining the degree of genetic characterization performed on cell lines and on preclinical model systems used in our published papers. We welcome a discussion on this topic, and on how we may best serve our community in this regard.