Please cite this article in press as: Parker and Perou, Tumor Heterogeneity: Focus on the Leaves, the Trees, or the Forest?, Cancer Cell (2015), http://dx.doi.org/10.1016/j.ccell.2015.07.011

CellPress

Tumor Heterogeneity: Focus on the Leaves, the Trees, or the Forest?

Joel S. Parker[1,2] and Charles M. Perou[1,2,*]
[1]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA
[2]Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA
*Correspondence: cperou@med.unc.edu
http://dx.doi.org/10.1016/j.ccell.2015.07.011

Molecular studies of intra-tumor heterogeneity (ITH) are revealing a large amount of DNA-based variation in many individual tumors. In a recent publication by Yates and colleagues, the ITH of breast tumors was examined and shown to have important implications for the development of resistant disease, metastasis, and clinical sequencing efforts.

Human breast cancer shows great inter-tumor diversity in every feature that one might examine, including histology, response to therapy, and patient survival times. At the same time, there is a long history of evidence for significant intra-tumor heterogeneity (ITH), which can itself be manifested in histological differences, cellular differences, and molecular differences. In fact, studies on ITH as assessed at the DNA level have been documented and studied for decades (Kallioniemi, 1988). The new study by Yates et al. (2015) masterfully brings these ITH DNA-based analyses into the 21st century, providing an unprecedented look at the sub-clonal composition of individual breast tumors.

The first question addressed by the authors was the amount of spatial ITH seen within individual untreated primary tumors through in-depth analysis of 12 patients in which each tumor was sampled from 8 different locations. Each location was then subjected to deep sequencing using a 300-gene panel. Two thirds (i.e., 8 of 12) of the tumors showed clear somatic mutation heterogeneity across the different isolates from a single tumor; 2 of 12 showed copy number ITH; and 2 of 12 showed little genetic variation. Thus, the majority of tumors showed molecular ITH, and, in most tumors, this was spatially segregated. This characterization of spatial ITH supports the findings of others (Ding et al., 2010; Shah et al., 2012), but with a larger number of breast tumors. Finally, the authors highlight 4 of 12 cases in which the sub-clonal mutations included important genes like *TP53*, *PIK3CA*, and *BRCA2*. This finding dictates the necessity to sequence at high-depth in the clinical setting to generate sensitive and specific sub-clonal mutation calls.

The finding that oncogenic mutations can be sub-clonal prompts the question of whether these were present at diagnosis or if they occurred later during tumor evolution. Previous work in breast cancer has shown that, within triple-negative breast cancer (TNBC), *TP53* mutations are frequent and tend to be early events (Ding et al., 2010; Shah et al., 2012). Yates et al. (2015) extend this work using four patients that presented with multi-focal disease (i.e., two to five foci per patient). In each case, foci were clearly genetically related to the others within a patient but also had many private mutations that were present at high variant allele fractions (VAF). The authors infer that individual foci must have arisen from a common ancestor, and thus their spatial separation was almost akin to local metastasis. Second, each foci must have undergone a "clonal sweep", evident by numerous private mutations with high VAF. These results are in line with those of other researchers (Navin et al., 2011; Nik-Zainal et al., 2012), supporting the concept of "clonal sweep" in breast cancer. Another result of this work was some evidence that groups of genetic variants are present in the metastasis at high VAF and in the primary tumor at low VAF, similar to what was recently described in prostate (Gundem et al., 2015). Further work is needed to understand if metastatic sites are typically seeded from a single clone or a more heterogeneous population of cells.

Yates et al. (2015) also touched briefly on comparisons of primary tumors versus residual disease after neoadjuvant chemotherapy. In 5 of 18 cases, they found evidence for the emergence of sub-clones not originally detected before chemotherapy. The notion of tumor evolution (through continual acquisition of new mutations or punctuated clonal expansion) will be important to understand for clinical studies. At the least, these results strongly argue that diagnostic specimens for DNA-based assays should sample the tumor as close to the time of the new treatment as possible. Otherwise, it is plausible that there are differences in the residual disease or metastatic site that do not represent the earlier diagnostic specimen. In such cases, test results may lead to sub-optimal therapeutic decisions.

The authors also hypothesized that a quantitative measure of ITH, based upon somatic DNA variation, might have clinical implications, and thus they developed an "index of heterogeneity". No associations with estrogen receptor (ER) status, grade, lymphocyte infiltration, tumor Ki-67 score, or response to chemotherapy were observed across the complete cohort. The finding of no association between the index of heterogeneity and clinical features may be evidence that clonal diversity itself is not meaningful. However, the lack of association may be due to limited power due to the small sample size (n = 50) and diverse composition of the cohort (27 ER^+, 3 $HER2^+$, and 20 TNBC). Regardless, assessments of ITH are of clear biological value, and we believe (like Yates and colleagues) that measures of ITH will ultimately aid our understanding and treatment of cancers.

Molecular assessments of ITH are now in the translation period from the research

setting into the clinical setting, with the ultimate question being what their clinical utility is. For common clinical use, an assay must achieve analytical validation and clinical validation and ultimately demonstrate clinical utility. Measurement of ITH, and future trials with ITH, will need to be developed in all three areas. In terms of analytical validity, sequencing depths and computational protocols for reconstructing clonal structure are still being developed and will need to be optimized. In terms of clinical validity, recent work has shown that exome-based measurement of ITH may predict outcomes in head and neck cancer (Mroz et al., 2015), and it seems intuitive that tumors with greater ITH would harbor more genetic diversity from which drug resistant sub-clones could emerge.

Lastly, the clinical utility of ITH as a biomarker has yet to be demonstrated, and it is on this front where the unanswered questions are the most clinically challenging. These questions include the prognostic potential of ITH, while biologically plausible and with good preliminary evidence, must be weighed against numerous well-developed prognostic biomarkers. Second is the question of what to do with a ''clinically actionable'' variant when it is sub-clonal. For example, a case where standard practice sequencing of a lung adenocarcinoma identifies a sub-clonal (i.e., 15% VAF) EGFR L858R mutation. Should the EGFR tyrosine kinase inhibitor (TKI) inhibitor be given even when the majority of the tumor cells do not contain the mutation? The breast cancer ASCO/CAP guidelines state that sub-clonal (i.e., not present within all tumor cells) HER2 high protein expression and/or amplification or ER protein expression indicates treatment with anti-HER2 and anti-estrogens agents, respectively (Hammond et al., 2010; Wolff et al., 2007). Such indications are dictated by trial criteria, and, in this context, it appears that the community is in agreement that targeted agents should be considered in sub-clonal disease.

A more difficult question arises when sub-clonal resistance mutations are identified. For example, consider the case of a lung cancer at diagnosis with a L858R EGFR mutation with high VAF and a sub-clonal T790M resistance mutation (i.e., 1%–5% VAF). This situation is observed in the clinic and poses the very difficult question of whether to prescribe the standard of care (i.e., first-generation EGFR TKIs) or to jump to the drugs that target the T790M and L858R EGFR mutation variants. These are already challenges being faced in lung cancer and chronic myeloid leukemia with drugs targeting BCR-ABL. The study by Yates and colleagues contributes to these efforts by providing a new and powerful means of assessing ITH and a means of assessing tumor branching evaluation. Their findings impact clinical study design in breast cancer and could soon impact clinical practice.

REFERENCES

Ding, L., Ellis, M.J., Li, S., Larson, D.E., Chen, K., Wallis, J.W., Harris, C.C., McLellan, M.D., Fulton, R.S., Fulton, L.L., et al. (2010). Nature *464*, 999–1005.

Gundem, G., Van Loo, P., Kremeyer, B., Alexandrov, L.B., Tubio, J.M., Papaemmanuil, E., Brewer, D.S., Kallio, H.M., Högnäs, G., Annala, M., et al.; ICGC Prostate UK Group (2015). Nature *520*, 353–357.

Hammond, M.E., Hayes, D.F., Dowsett, M., Allred, D.C., Hagerty, K.L., Badve, S., Fitzgibbons, P.L., Francis, G., Goldstein, N.S., Hayes, M., et al. (2010). J. Clin. Oncol. *28*, 2784–2795.

Kallioniemi, O.P. (1988). Cytometry *9*, 164–169.

Mroz, E.A., Tward, A.D., Hammon, R.J., Ren, Y., and Rocco, J.W. (2015). PLoS Med. *12*, e1001786.

Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., Cook, K., Stepansky, A., Levy, D., Esposito, D., et al. (2011). Nature *472*, 90–94.

Nik-Zainal, S., Van Loo, P., Wedge, D.C., Alexandrov, L.B., Greenman, C.D., Lau, K.W., Raine, K., Jones, D., Marshall, J., Ramakrishna, M., et al.; Breast Cancer Working Group of the International Cancer Genome Consortium (2012). Cell *149*, 994–1007.

Shah, S.P., Roth, A., Goya, R., Oloumi, A., Ha, G., Zhao, Y., Turashvili, G., Ding, J., Tse, K., Haffari, G., et al. (2012). Nature *486*, 395–399.

Wolff, A.C., Hammond, M.E., Schwartz, J.N., Hagerty, K.L., Allred, D.C., Cote, R.J., Dowsett, M., Fitzgibbons, P.L., Hanna, W.M., Langer, A., et al.; American Society of Clinical Oncology/College of American Pathologists (2007). Arch. Pathol. Lab. Med. *131*, 18–43.

Yates, L.R., Gerstung, M., Knappskog, S., Desmedt, C., Gundem, G., Van Loo, P., Aas, T., Alexandrov, L.B., Larsimont, D., Davies, H., et al. (2015). Nat. Med. *21*, 751–759.