NEWS

Many Moving Parts

The linker, a small organic chemical moiety or peptide of several amino acids that secures the antibody to the toxin, is important, said Amit K. Verma, M.B., B.S., associate professor of medicine at the Albert Einstein College of Medicine in New York. Because the ADC must travel through the bloodstream to reach its target without breaking apart, an ADC is only as good as the linker. Linkers need to be stable in the bloodstream (ADCs can circulate for as long as a few weeks) but must break down once inside the cell, where the linker is cleaved by enzymes or undergoes a change in pH to release the toxin. This is a fine balance. A linker that is too stable can result in minimal antitumor activity and toxic effects.

"You don't want the toxin portion of the ADC to be hanging around in the circulation for too long," said Verma. "The toxin in circulation can result in vascular leak and other toxic effects."

This small peptide needs to be stable also because only a tiny fraction of the ADC molecules—about 1%–5%—will end up binding to tumor cells. The rest are metabolized, preferably to a less toxic version, to minimize potential toxic effects. For T-DM1, "the metabolite that is released into the liver was found to be less toxic than the original toxin that was conjugated," said Gerber. According to Gerber, the site on the antibody where the linker is attached also plays a role in the safety profile of these macromolecule drugs.

Toxic Payload

The toxins in current ADCs target either DNA or tubulin, the two main targets of chemotherapy. "ADCs are very selective, but they are not very efficient ways to deliver chemotherapy, so these drugs need to be very potent agents," said Teicher. Several toxins currently incorporated into ADCs originally failed as stand-alone chemotherapy drugs: They were too toxic.

Much ADC research focuses on developing toxins, either as derivates of natural compounds or new molecules. The toxins must be able to bind to the linker and be stable in the three-part complex in the blood. ADCs that work on solid tumors can benefit from toxins that, when liberated from inside the dying cell, can slip through the membranes of adjacent tumor cells, which need not express the targeted antigen. Like tinker toys, some antibody, linker, and toxin combinations allow multiple linker–toxin pairs to be attached to an individual antibody, lowering the ADC concentration required for tumor cell death, and probably the toxicity.

Just as for other targeted cancer agents, research efforts to find new ways to identify patients who could benefit from ADCs are ongoing. Although immunohistochemistry is still the "gold standard" to detect whether a tumor expresses a specific target, detecting the antigen on circulating tumor cells (CTCs) may be a noninvasive approach to assess whether a patient will respond to the ADC. During the clinical development stage, expression on CTCs, associated with patient response, can identify the threshold of expression on CTCs needed for a clinical response.

With many clinical programs to create ADCs, much more is now understood about both the efficacy and safety of these drug types than was true even just a few years ago. "We can now connect the dots better from the preclinical studies to more reliably predict what will or will not be seen in the clinic with these agents," said Gerber.

The current burst of activity to develop more-effective and better-tolerated ADCs in the pharmaceutical industry is thanks to the research supported by NCI in the last few decades, said Weiner. "We are world leaders in this exciting technology, and the reason is that we invested in basic and applied research," said Weiner. "This is a great example of how investment in biomedical research can show a great long-term payoff."

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Genetic Events in Head and Neck Squamous Cell Carcinoma Revealed

Nancy J. Nelson

Researchers at April's annual meeting of the American Association for Cancer Research in Washington, D.C., announced a complete analysis of the genetic alterations of head and neck squamous cell carcinoma (HNSCC). "This is really a parts list for head and neck cancer," said David Neil Hayes, M.D., M.P.H., an associate professor at the University of North Carolina at Chapel Hill who sees HNSCC patients at the UNC Lineberger Comprehensive Cancer Center. Hayes is senior author of the February *PLoS ONE* article that highlights some of the findings. "There hasn't been such a list available until now."

The study is part of The Cancer Genome Atlas (TCGA) project supported by the National Institutes of Health to catalog genomic changes in cancers with poor prognosis or a major impact on public health. The data are then made publicly available. Thirty cancers have been selected; about one-third are complete, whereas the others are in various stages of sample collection or data analysis (https://tcga-data.nci.nih.gov/tcga/). HNSCC is the sixth-most-common cancer worldwide, with about 600,000 new cases every year, and includes cancer of the nose cavity,

sinuses, lips, mouth, salivary glands, throat, and voice box. About half of patients survive 5 years after diagnosis. Radiation, chemotherapy, surgery, and cetuximab (which inhibits epidermal growth factor receptor [EGFR]) are treatments for HNSCC. Major risk factors include smoking, alcohol consumption, and human papillomavirus (HPV) infection.

Hayes and colleagues analyzed tumor and healthy tissue from 279 people with HNSCC. Over 6 years, the researchers collected data on DNA, RNA, and miRNA sequencing; RNA expression; promoter methylation; and protein arrays.

Analysis revealed four subtypes of cancer, named according to type of genes expressed in each subtype: basal, mesenchymal, classical, and atypical. The subtypes do not correspond to patient age, race, sex, tobacco use, or organ site but are very similar to subtypes found in a recent TCGA analysis of 178 tumor samples of lung squamous cell carcinoma. For example, both basal subtypes show high expression of COL17A1 associated with extracellular matrix, growth factor TGFA, growth receptor EGFR, and transcription factor TP63. Mesenchymal subtypes in both organs overexpress VIM and DES, transcription factor TWIST1, and growth factor HGF.

Finding similar subtypes in cancers of both the lung and the head and neck suggests that common cancer pathways are at work across anatomic sites. Hayes speculates that future successful therapies might more rapidly be applied to a wider set of cancers rather than chipping away at cancer one anatomic site at a time.

DNA sequencing revealed several genes mutated at rates higher than background, considered "driver" cancer genes: CDKN2A, FAT1, TP53, CASP8, PIK3CA, TGFBR2, HRAS, RB1, KEAP1, NFE2L2, B2M, and RAC1.

The researchers also compiled a list of 18 candidate therapeutic targets, genes for which drugs are already available. Four of these targets are considered driver genes—CDKN2A, PIK3CA, TP53, and HRAS—and half are amplified receptor tyrosine kinases, such as EGFR, FGFR1 and 2, MET, and ERBB2.

Addressing the prevalence of the targets, Hayes pointed out that some targets are rare and not worth pursing, whereas others, such as FAT1, FGFR, and CCND, are highly amplified and, until now, underappreciated. "One of the vitally important findings of the current study is that EGFR is not amplified in HPV+ tumors, which make up about 15% of HNSCC tumors," said Hayes. "The clinical confirmation is lacking, but it raises serious questions about the use of EGFR inhibitors in some tumors." Rather than treat HPV+ tumors with an EGFR inhibitor, the data suggest that a more appropriate target may be PIK3CA, a gene with a high mutation rate in HPV-infected cells.

Hayes is excited at the quantity of data available to mine. "We've never had large data sets to work with. Now that we have the same groups of researchers with the same high-quality samples doing the same analysis, we have been able to streamline the process."

He thinks "Google Earth" of Cancer Genome is a more appropriate name than The Cancer Genome Atlas. "You can zoom all the way out to 10,000 feet and look at 50 tumors all at once, and then zoom in on a neighborhood and compare one chromosome in one tumor to another chromosome in a neighboring tumor. You get to see the world of a tumor in totally different light."

"We've never had large data sets to work with. Now that we have the same groups of researchers with the same high-quality samples doing the same analysis, we have been able to streamline the process."

One researcher who has begun to mine the data is Christine Chung, M.D., associate professor of oncology at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center in Baltimore, who has been studying HNSCC for many years. Given that no good treatment options or personalized therapies exist for recurrent or metastatic HNSCC, Chung thinks that these data will help to identify patients at risk for poor survival at time of diagnosis and improve current treatments based on molecular genetic aberrations. Although the U.S. Food and Drug Administration has approved cetuximab to treat HNSCC, the drug has a response rate of about 10% when used as a single agent in recurrent/metastatic disease. Most of the TCGA data did not surprise Chung, because two sets of investigators (led by Agrawal and Stransky) reported in 2011 in *Science* several of the mutated genes found in the TCGA study. However, the *PLoS ONE* study reports several new findings such as elevated mutation rates of KEAP1 and NFE2L2, involved in the oxidative stress pathway. She has begun to download some data to try to identify dominant pathways in each cancer subtype. Then she can begin developing potential drugs with an eye to doing a clinical trial with all four subtypes.

David Neil Hayes, M.D., M.P.H.

Scott Lippman, M.D., director of the Moores Cancer Center at the University of California, San Diego, agrees that the TCGA data validate and expand on the smaller studies. The mutation rates, for example, of several genes in the Agrawal and Stransky studies, involving 32 and 74 patient tumors, respectively, are similar to TCGA rates. These genes include NOTCH1, FAT1, TP53, CASP8, and PIK3CA. Also, all three studies reported lower mutation rates in HPV-infected tumors than in noninfected ones, very high mutation rates of TP53, and many more mutations in heavy smokers than in nonsmokers.

The TCGA data offer a complete catalogue of the mutations and their frequency. "Now," said Lippman, "before testing in clinical trials, we need to do preclinical studies. This means functionally characterizing the biological pathways affected by the newly identified mutations so that we can screen and test for small molecules and other inhibitors that target members of the pathways."

His group is trying different cell systems to look at NOTCH1, FAT1, and CASP8. Unfortunately, some highly mutated genes in HNSCC are inactivated tumor suppressor genes, such as NOTCH1, TP53, and CDKN2A. This setting may require synthetic lethal or other approaches, which are more complicated than creating a small-molecule inhibitor or an antibody to inhibit activated oncogenes.

Although many researchers are focusing on gene mutations, others are analyzing miRNA, epigenetic data, and protein arrays to dissect molecular pathways. Chung thinks the scope of the TCGA data is its real strength. "We have comprehensive knowledge of the tumor genome now, including DNA mutations, copy number variation, gene and protein expression, and epigenetic changes. That's the most valuable data that we have ever collected. You can follow entire molecular changes from DNA to protein. That's why these data are so important to the scientific community."

So how is the scientific community reacting to the volume of tumor data accumulating on TCGA's Data Portal and Cancer Genomics Hub? The head and neck data represent the latest addition, which already includes similar data for breast, colorectal, ovarian, adenocarcinoma and squamous cell carcinoma of the lung, glioblastoma, and endometrial cancers.

An analysis on the TCGA website shows that from January 2011 to December 2012, the number of visitors to TCGA's Data Portal more than doubled, from 3,386 to 8,267. The number of grant applications that propose using TGCA data has also increased: In 2006, only such 46 such grants were submitted, compared with 800 in 2012. And, finally, the number of published papers that analyze TCGA data increased from three in 2008 to 350 by May 2013. These papers, including the tumor-specific marker papers written by the TCGA Research Network, are available online (http://cancergenome.nih.gov/publications/). Hayes expects to add his publication by early 2014.

As researchers and drug companies tap into the massive data sets over the years, the secrets of the tumors will gradually unravel. That is already happening with the cancers analyzed earlier:

- Researchers from the University of Texas M. D. Anderson Cancer Center in Houston using TCGA ovarian cancer data may have identified a miRNA important in preventing metastasis.
- Scientists at Columbia University found a fusion gene product in some TCGA glioblastoma multiforme tumors that seems to be a marker of aggressiveness. When the tumors were treated in animals with an inhibitor to one of the genes, survival was prolonged.
- Yet another group from Hong Kong using TCGA RNA expression profiling from glioblastoma tumors discovered that six long noncoding RNAs may predict survival.

And that's just the beginning.

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PDQ (Physician Data Query) *is the National Cancer Institute's source of comprehensive cancer information. It contains peer-reviewed, evidence-based cancer information summaries on treatment, supportive care, screening, prevention, genetics, and complementary and alternative medicine. The summaries are regularly updated by six editorial boards. The following PDQ summaries were recently updated:*

Souza FH, Wendland EM, Rosa MI, et al.: Is full-field digital mammography more accurate than screen-film mammography in overall population screening? A systematic review and meta-analysis. Breast 22 (3): 217–24, 2013. PMID: 23489759

The **PDQ Breast Cancer Screening summary** was recently updated to include results from a meta-analysis comparing digital mammography with film mammography. The meta-analysis included 10 studies and involved 82,573 women who underwent both types of mammography. In a random-effects model, there was no statistically significant difference in cancer detection between the two types of mammography (AUC 0.92 for film and AUC 0.91 for digital). For women younger than 50 years, all studies found sensitivity higher for digital mammography but specificity either the same or higher for film mammography. In a large U.S. cohort study that was included in the meta-analysis, sensitivity for women younger than 50 years was 75.7% (95% CI, 71.7–79.3) for film mammography and 82.4% (95% CI, 76.3–87.5) for digital mammography; specificity was 89.7% (95% CI, 89.6–89.8) for film and 88.0% (95% CI, 88.2–87.8) for digital. The meta-analysis found no other differences by age.

Domchek SM, Jhaveri K, Patil S, et al.: Risk of metachronous breast cancer after *BRCA* mutation-associated ovarian cancer. Cancer 119 (7): 1344–8, 2013. PMID: 23165893

Vencken PM, Kriege M, Hooning M, et al.: The risk of primary and contralateral breast cancer after ovarian cancer in *BRCA1/BRCA2* mutation carriers: Implications for counseling. Cancer 119 (5): 955–62, 2013. PMID: 23165859

The **PDQ Genetics of Breast and Ovarian Cancer summary** was recently updated to include results from two genetic registry-based studies that explored the risk of primary breast cancer after BRCA-related ovarian cancer. In one study, 164 BRCA1/2 mutation carriers with primary epithelial ovarian, fallopian tube, or primary peritoneal cancer were followed for subsequent events. The 5-year and 10-year breast cancer-free survival rates were 97% (95% CI, 0.92–0.99) and 91% (95% CI, 0.82–0.95), respectively. In this series, overall survival was dominated by ovarian cancer-related deaths. A similar study compared the risk of primary breast cancer in BRCA-related ovarian cancer