## Clinical Trials

**Major finding:** A proof-of-principle trial shows that adding immunotherapy to radiotherapy can elicit abscopal responses.

**Concept:** An abscopal response in a nonirradiated metastatic site may be predictive of a better outcome.

**Impact:** These findings support further evaluation of radiotherapy in combination with immunotherapeutic agents.

### COMBINING GM-CSF WITH RADIOTHERAPY INDUCES ABSCOPAL RESPONSES

Radiotherapy can induce regression of distant nonirradiated tumors, a phenomenon known as an abscopal response. Preclinical studies indicate that abscopal responses are mediated by radiotherapy-induced immune responses, prompting Golden and colleagues to evaluate whether the combination of local radiotherapy and the immune adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) would lead to abscopal responses in patients with metastatic tumors receiving chemotherapy or hormonal therapy. Patients with stable or progressive disease were enrolled in a proof-of-principle trial to determine whether concurrent radiotherapy to one metastatic lesion and daily subcutaneous injection of GM-CSF would induce an abscopal response, defined as a greater than 30% decrease in the longest diameter of any nonirradiated lesion. The primary endpoint of the trial was to determine the proportion of patients with an abscopal response, and the secondary endpoints were to evaluate safety and overall survival. Monitoring of immune cells in complete blood counts was added as an exploratory endpoint. Of 41 patients enrolled, 11 (26.8%) experienced an abscopal response, and patients who had an abscopal response had significantly better overall survival compared with those who did not (20.98 months vs. 8.33 months). The combination regimen was well tolerated; although some adverse events were observed, no patient required a dose reduction or needed to discontinue treatment. The exploratory analysis revealed that abscopal responders had a significantly lower baseline median neutrophil-to-lymphocyte ratio than nonresponders, raising the possibility that neutrophils may suppress radiotherapy-induced immune responses and that this ratio might be useful for predicting response to combined radiotherapy and immunotherapy, though prospective longitudinal immunomonitoring is needed. Although a contribution of systemic therapies to the abscopal responses cannot be ruled out, these findings provide evidence that combining immunotherapy with radiotherapy can induce abscopal responses, and provide a rationale for the further clinical evaluation of radiotherapy in combination with immunotherapies in patients with metastatic cancer. ■

*Golden EB, Chhabra A, Chachoua A, Adams S, Donach M, Fenton-Kerimian M, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: a proof-of-principle trial. Lancet Oncol 2015;16:795–803.*

## Breast Cancer

**Major finding:** Primary breast cancer exhibits variable spatial and temporal patterns of subclonal diversification.

**Approach:** Multiregion sequencing characterized subclonal growth, heterogeneity, and tumor evolution.

**Impact:** Multiregion analysis is required to optimize design of clinical trials and therapeutic strategies.

### MULTIREGION SEQUENCING DEFINES SUBCLONAL STRUCTURE IN BREAST CANCER

Past sequencing efforts highlighting subclonal evolution and intratumor heterogeneity in breast cancer emphasize the challenge of using single tumor region analysis to direct therapeutic strategies. To address whether breast cancer follows a temporal order of somatic mutation accumulation and geographic stratification of clonal structure, Yates and colleagues performed whole-genome and targeted sequencing of multiple tumor regions from 50 invasive breast cancers, including treatment-naïve and paired pretreatment and post-chemotherapy samples, for a total of 303 samples. Sequencing of spatially distinct regions within 12 tumors revealed mutational or copy-number heterogeneity in 10 tumors; the majority of tumors were characterized by restricted localization of subclones, whereas some tumors exhibited broad subclonal mixing. Sequencing of multifocal cancers suggested several patterns of growth, including foci-specific alterations in driver genes, related subclonal populations present in geographically distinct foci, and subclonal dissemination. Both targeted capture and whole-genome sequencing methods highlighted variable levels of genetic heterogeneity among tumors. Sequencing of pre- and post-chemotherapy biopsies revealed that resistance mechanisms were likely acquired in pretreatment subclones at very low frequency. Similarly, sequencing showed that metastatic lesions were often seeded by primary tumor subclones, suggesting that targeting subclonal mutations in the primary tumor may help to prevent disease progression. Alterations in common driver genes were observed both early and late in tumor evolution, and a subset of cancers displayed parallel evolution of subclonal driver mutations, which often cooperated with trunk mutations to disrupt tumor suppressor gene function. In addition, structural variants, including tandem duplications and complex chromosomal rearrangements, were shown to drive late-stage subclonal evolution in some cases. Together, these data emphasize the intratumor complexities of breast cancer subclonal diversification and reinforce the necessity of multiregion sequencing analysis to direct future clinical trials. ■

*Yates LR, Gerstung M, Knappskog S, Desmedt C, Gundem G, Van Loo P, et al. Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 2015;21:751–9.*