Research Highlights

Highlights from the latest articles in biomarkers

Backtracking on CYP2C19 genotyping in clopidogrel therapy?

Evaluation of: Pare G, Mehta SR, Yusuf S *et al.*: Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. *N. Engl. J. Med.* doi: 10.1056/NEJMoa1008410 (2010) (Epub ahead of print).

This recent article casts doubt on the use of CYP2C19 genotyping as a marker for determining the use of the platelet inhibitor clopidogrel (Plavix®) in patients with cardiovascular disease. Over the past couple of years, a series of high-profile articles have created a paradigm that clopidogrel is not as effective and results in worse clinical outcomes in cardiovascular disease patients with genotypes associated with loss of function in CYP2C19. CYP2C19 converts clopidogrel to its active metabolite; therefore, loss of function of CYP2C19 results in decreased inhibition of platelet activity. There was sufficient clinical evidence that CYP2C19 loss-of-function genotypes resulted in decreased clopidogrel inhibition of platelet activity that in March 2010 the FDA issued a black-box warning for clopidogrel with the recommendation for alternative dosing in patients with loss-of-function CYP2C19 genotypes.

The current article in the *New England Journal of Medicine* investigated the genotype of patients from two previous randomized clinical trials: Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) and Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A (ACTIVE A). These two clinical trials had previously shown that, compared with placebo, clopidogrel significantly reduced cardiovascular death in patients with acute coronary syndrome (CURE)

and significantly reduced major vascular events in patients with atrial fibrillation (ACTIVE A). In the *New England Journal* study, all patients were genotyped for three major single-nucleotide polymorphisms: CYP2C19*2, CYP2C19*3 and CYP2C19*17. This study found that a CYP2C19 loss-of-function genotype status did not predict the performance of clopidogrel in either the CURE or ACTIVE A trial patients. The effect of clopidogrel on bleeding was not significantly different between the wild-type and loss-of-function genotypes. Overall, CYP2C19*2, *3 and *17 single-nucleotide polymorphisms were not predictive in determining the efficacy of clopidogrel. A possible explanation for the departure from the previously established paradigm was proposed by the authors of the *New England Journal* study: the CURE trial only had 18% of patients who underwent percutaneous coronary intervention, and only 14.5% of patients had a coronary artery stent placed. This is compared with prior CYP2C19 studies that had coronary stenting in 70% of patients. In the current analysis, no difference was found in cardiovascular events by CYP2C19 genotype in patients with or without stenting. However, the authors of the current study could not rule-out the possibility of an interaction in a subgroup of patients, and, in particular, they note that patients in the CURE trial did not receive drug-eluting stents.

The findings of the *New England Journal* study are not sufficient to eliminate the need for CYP2C19 genotyping in all settings of clopidogrel therapy, but they are a reminder that rationale drug therapy based on biomarkers is not perfect. Both the biomarkers and drugs used must both be thoroughly validated by well designed, prospective randomized controlled trials.

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Melanoma-targeted therapy based on V600E *BRAF* mutation

Evaluation of: Flaherty KT, Puzanov I, Kim KB *et al.*: Inhibition of mutated, activated *BRAF* in metastatic melanoma. *N. Engl. J. Med.* 363(9), 809–819 (2010).

Activating mutations of *BRAF* occur frequently in melanomas with a reported prevalence of between 30 and 70%. This particular study examined the clinical utility of a small molecule inhibitor specific for the V600E mutation. The orally available drug PLX4032 (Plexxikon; RG7204, Roche Pharmaceuticals) had been previously shown to have tumor-specific effects in animal models. The present study was a multicenter Phase I dose-escalation trial followed by a Phase II extension at the maximally tolerated dose. Phase I enrollment did not require a V600E mutation

in the tumor and was not restricted to patients with melanoma. Phase II only enrolled melanoma patients with the V600E mutation. V600E mutation status was determined by a PCR specific for the point mutation.

In Phase I, 11 of 16 melanoma patients with the V600E mutation had partial or complete response; ten patients had partial response and one had a complete response. In Phase II, 26 of 32 melanoma patients with V600E mutation had partial or complete response; 24 patients had partial response and two had complete response. More than 5% of the 87 study patients had an adverse drug-related event of grade 2 or higher. The most common dose-limiting toxicities were rash, fatigue or arthralgia.

The importance of V600E mutation-directed therapy is suggested by the five

patients in Phase I that had metastatic melanoma without *BRAF* mutation. None of these five patients had tumor regression, and four of these patients had progression during the first 2 months of PLX4032 treatment. The results of this trial of PLX4032 are exciting because 81% of patients with V600E-mutated melanoma had partial or complete regression. Response to therapy was seen even at non-maximal doses in Phase I and was observed in all sites of metastatic disease (e.g., bone, liver and small bowel). This response to PLX4032 was specific to tumors with the V600E mutation. Indeed, three patients in Phase I with papillary thyroid cancer with the V600E mutation had partial or complete response. PLX4032 is now being evaluated in a Phase III trial to determine if there is an improvement in overall survival.

Tumor-specific therapy based on *BRCA1/2* mutation status

Evaluation of: Tutt A, Robson M, Garber JE *et al.*: Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 376, 235–244 (2010); Audeh MW, Carmichael J, Penson RT *et al.*: Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* 376, 245–251 (2010).

Mutations in *BRCA1* or *BRCA2* are well described in hereditary breast and ovarian cancer. Recently, therapeutics that specifically target BRCA-deficient or -mutated cells have made it into clinical trials.

Olaparib (AZD2281, AstraZeneca) is an orally active poly(ADP-ribose) polymerase (PARP) inhibitor. PARP inhibitors target BRCA-deficient or -mutated cells by exploiting the same mechanism by which BRCA defects result in tumors. *BRCA1* and *BRCA2* are involved in DNA repair; when they are mutated there is an increase in genomic instability that leads to neoplastic transformation and tumor formation. PARP inhibition results in the accumulation of dsDNA breaks that can be repaired by wild-type *BRCA1* or *BRCA2*, but in cells with mutated *BRCA1* or *BRCA2*, the dsDNA breaks cannot be repaired and cell death occurs. Thus, PARP inhibitors drive cells with *BRCA1* or *BRCA2* mutations into cell death.

The promise of the PARP inhibitor AZD2281 as an anti-tumor agent was exhibited in the Phase I trial that included

patients that were carriers for mutations in *BRCA1* or *BRCA2* [1]. This initial trial demonstrated lower toxicity compared with conventional chemotherapy and there was objective anti-tumor activity of AZD2281 in patients that were carriers of *BRCA1* or *BRCA2* mutations.

Phase II trial results for AZD2281 are now published in back-to-back articles in *Lancet*. The first article [2] reports the activity of PARP inhibitor monotherapy in patients with *BRCA1* or *BRCA2* mutations and advanced stage breast cancer. The patients in this trial had previously received a median of three chemotherapy regimens. The cohort that received the maximum dosage of 400 mg twice daily had an objective response rate of 41% (11 of 27 patients) with one report of complete response. The second article [3] reports the activity of AZD2281 as a monotherapy in

patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer. Similar to the advanced breast cancer trial, the patients had previously received a median of three chemotherapy regimens. The cohort in the recurrent ovarian cancer trial that received the maximum dosage of 400 mg twice daily had an objective response rate of 33% (11 of 33 patients) with two reports of complete response. For both *Lancet* articles, the reported safety and tolerability were comparable to the Phase I trial with low grade toxicities reported.

BRCA mutational status is often regarded as a risk factor for future malignancy, but has not until recently been considered

a biomarker for predicting response to pharmacological therapy. The Phase II data for the PARP inhibitor AZD2281 in advanced breast cancer and recurrent ovarian cancer is promising. In both Phase II studies, PARP inhibitor monotherapy showed objective response in patients that had failed multiple chemotherapy regimens. The next step will be examining whether PARP inhibition impacts overall survival.

References

- 1 Fong PC, Boss DS, Yap TA *et al.*: Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. *N. Engl. J. Med.* 361, 123–134 (2009).
- 2 Tutt A, Robson M, Garber JE *et al.*: Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 376, 235–244 (2010).
- 3 Audeh MW, Carmichael J, Penson RT *et al.*: Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* 376, 245–251 (2010).

Trastuzumab for HER2-positive gastric and gastroesophageal junction cancers

Evaluation of: Ban YJ, Cutsem EV, Feyereislova A *et al.*: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of *HER2*-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a Phase III, open-label, randomized controlled trial. *Lancet* 376, 687–697 (2010).

The use of Herceptin® (trastuzumab) monoclonal antibody therapy against breast cancers overexpressing *HER2* is one of the leading examples of personalized medicine for oncology. The results of a recent multinational Phase III trial may expand the

use of trastuzumab to gastric cancers. The Trastuzumab for Gastric Cancer (ToGA) trial examined the use of trastuzumab in combination with standard chemotherapy for the treatment of *HER2*-positive gastric and gastroesophageal junction cancers. The inclusion criteria for patients were *HER2* positivity as determined by immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH). The *HER2* IHC and FISH tests used were commercially available tests currently used for breast cancers (HercepTest, Dako, Denamrk, and *HER2* FISH pharmDx, Dako). These tests were performed in a centralized laboratory and were interpreted using criteria specific for gastric cancers. Of the 3803 patients assessed for trial eligibility, 3665 had an

interpretable *HER2* result by IHC or FISH. *HER2* positivity was demonstrated in 22% of the patients (810 out of 3665). The median overall survival for patients treated with trastuzumab in combination with chemotherapy was 13.8 months compared with 11.1 months for patients treated with chemotherapy alone. The rate of severe adverse events was not significantly different between the two treatment groups.

Based on the results of the Phase III ToGA trial, trastuzumab has now been submitted to the US FDA for consideration as a new option for treating gastric or gastroesophageal cancers. Importantly, this is an example of expansion of a drug indication based on predictive biomarker testing (*HER2* positivity).