Can sensitivity to cytotoxic chemotherapy be predicted by biomarkers?

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Non-small-cell lung cancer is the leading cause of cancer-related death worldwide with poor outcomes, even when a curative treatment approach is feasible. Chemotherapy remains a key component of treatment in both the adjuvant and metastatic settings. Newer targeted therapies have demonstrated activity for advanced disease. However, many patients fail to achieve benefit with such treatments. Personalizing therapy to select those patients most likely to benefit promises to limit toxic effects and to maximize efficacy. A predictive biomarker is a patient or tumor characteristic that separates a population in regards to the outcome when using a particular treatment. To date, several molecular analyses and biomarkers have been studied. We review here the main predictive biomarkers to chemotherapy in both the adjuvant and the advanced settings.

In order to establish a methodological review of predictive biomarkers to chemotherapy in NSCLC patients, we have grouped them into six main categories: DNA synthesis/repair genes, cell cycle regulators, class III Beta-tubulin, signal transduction pathways, gene expression profiling and microRNAs.

DNA synthesis/repair genes

excision repair cross-complementation group 1

Excision repair cross-complementation group 1 (ERCC1) is a protein involved in the nucleotide excision repair pathway and interstrand cross-link repair pathway which has a key role in restoring DNA from platinum damage. Its value as a predictive biomarker in the adjuvant setting was analyzed in the IALT trial, patients with non-squamous stage II–IIIA NSCLC are randomized to standard cisplatin–pemetrexed (low ERCC1, low TS) or a taxane (high ERCC1, high TS). In the TASTE trial, patients with completely resected stage II–III NSCLC are treated with either a cisplatin-based doublet or a tailored treatment based on ERCC1 and TS levels. In the experimental arm, patients receive cisplatin–pemetrexed (low ERCC1, low TS), cisplatin–gemcitabine (low ERCC1, high TS), pemetrexed (high ERCC1, low TS) or a taxane (high ERCC1, high TS). In the TASTE trial, patients with non-squamous stage II–IIIA NSCLC are randomized to standard cisplatin–pemetrexed chemotherapy or to a customized arm (erlotinib for epidermal growth factor receptor (EGFR)-mutated patients, cisplatin–pemetrexed in EGFR wild-type/ERCC1-low patients or observation in EGFR wild-type/ERCC1-high/undetermined patients). In advanced NSCLC patients, several retrospective analyses demonstrated that high ERCC1 expression in the tumors, determined by RT–PCR or by IHC, was associated with poor response to platinum-based chemotherapy and shorter survival [2, 3]. In a Spanish Lung Cancer Group (SLCG) prospective trial in advanced disease, customized chemotherapy based on ERCC1 expression levels determined by RT–PCR compared with a standard chemotherapy resulted in a higher response rate but did not increase the length of survival [4]. Taken together, all these results are encouraging but further validation is needed before ERCC1 expression...
Median survival was 13.3 months, which suggests that high \(R_{RM1}\), low \(ERCC1\) or docetaxel–carboplatin use is a useful biomarker in the adjuvant or advanced setting.

**MutS homologue 2**

MutS homologue 2 (\(MSH2\)) is a protein which has a role in initiating DNA repair process in response to cisplatin-induced DNA damages [5]. Its predictive value with the use of adjuvant chemotherapy was studied within the IALT-Bio program [6]. In the low-\(MSH2\) group, assessed by IHC, there was a trend toward improving overall survival with adjuvant chemotherapy when compared with observation (adjusted HR for death: 0.76; 95% CI 0.59 to 0.97; \(P = 0.03\)). In the high \(MSH2\) group, there was no difference in the overall survival rate between the adjuvant chemotherapy arm and the observation arm. The effect of combining \(MSH2\) and \(ERCC1\) or \(p27\) expression was also explored. In the combined low \(MSH2/low ERCC1\) subgroup, the magnitude of the overall survival improvement with the use of adjuvant chemotherapy was greater than with any other low-expression marker alone (adjusted HR for death: 0.65; 95% CI 0.47 to 0.91; \(P = 0.01\)). In patients with advanced NSCLC, low \(MSH2\) expression detected by IHC predicts response to oxaliplatin-based chemotherapy (response rate 38% versus 0%, \(P = 0.04\)) [7].

**breast cancer gene 1**

Breast cancer gene 1 (\(BRCA1\)) is a protein with a central role in DNA repair which is also involved in cell cycle dynamics [8]. High \(BRCA1\) levels have been associated with platinum resistance in several models [9]. In resected NSCLC patients, high \(BRCA1\) mRNA levels by qRT–PCR seem to be a worse prognostic biomarker in chemotherapy-naïve patients [10]. Based on this finding, the SLCG is carrying out a prospective phase III randomized trial to evaluate \(BRCA1\) levels, assessed by qRT–PCR, as a predictive biomarker to address the use of adjuvant chemotherapy in completely resected stage II–III NSCLC patients [11]. In the control arm, patients receive cisplatin–docetaxel (Taxotere) and in the customized arm, patients with high \(BRCA1\) expression receive adjuvant docetaxel alone, those with low expression receive cisplatin–gemcitabine and those with intermediate expression receive cisplatin–docetaxel.

**Thymidylate synthase (TS)**

Thymidylate synthase (TS) is an important enzyme in the DNA replication and repair mechanisms, and it is also the main target of pemetrexed. The fact that TS expression level is higher in squamous cell carcinoma than in non-squamous cell carcinoma is one potential explanation for the sub-histologic-associated pemetrexed activity in NSCLC [14, 15]. The predictive value of TS in resected NSCLC is being studied in the ITACA trial.

**Cell cycle regulators**

**\(p27\)**

As a member of the cyclin-dependent kinase inhibitory family, its main activity consists in regulating progression from G1 to the S phase of the cell cycle. In completed resected patients, an important association between \(p27\) expression and benefit from adjuvant chemotherapy was observed in the IALT-Bio program [16]. Benefit derived from adjuvant chemotherapy was restricted to \(p27\)-negative tumors (HR 0.66, 95% CI 0.50 to 0.88; \(P = 0.006\)) [17].

**\(p53\)**

The tumor suppressor gene \(p53\) play a role in cell cycle, apoptosis induction and genome stability. In the JBR.10 trial, patients with high \(p53\) expression derived benefit from adjuvant chemotherapy (HR 0.54, 95% CI 0.32 to 0.92; \(P = 0.02\)) whereas patients with low \(p53\) expression did not. In contrast, \(p53\) mutations were not a predictive factor for adjuvant chemotherapy benefit [18]. The predictive value of \(p53\) expression and mutational status was also studied as part of the LACE-Bio program; where no significant interaction among \(p53\) expression or \(p53\) mutations and treatment was found [19].

**Bax**

Bax is a proapoptotic factor that is activated by \(p53\) [20]. Bax expression was studied in the IALT-Bio program. Bax expression showed a trend toward an association between increasing levels and higher sensitivity to adjuvant chemotherapy (HR 1.08, 0.83 and 0.68; for groups with low, medium and high Bax expression; \(P = 0.06\)) [21]. Bax expression was also evaluated in a pooled analysis of IALT and JBR.10 patients. In these analyses, Bax-positive patients derived benefit from adjuvant chemotherapy in comparison to observation (HR 0.72, 95% CI 0.56 to 0.91; \(P = 0.007\))[22].

**Class III beta-tubulin**

Microtubules are essential in cell dynamics and mitosis. Taxanes and vinca alkaloids disrupt normal microtubule function and cause mitosis arrest, which results in apoptosis. Class III beta-tubulin may mediate resistance to taxanes [23]. The value of class III beta-tubulin as a predictive factor was...
analyzed in a study in advanced NSCLC patients in which class III beta-tubulin levels were measured by qRT-PCR. In patients with low beta-tubulin levels, carboplatin/paclitaxel yielded a higher response rate than did cisplatin/vinorelbine or cisplatin/gemcitabine [24].

**signal transduction pathways**

**KRAS**
KRAS is a member of the RAS family, with an intrinsic GTPase activity. KRAS activation results in cell growth, differentiation and survival. KRAS mutations are present in up to 30% of lung adenocarcinomas. In the adjuvant setting, the KRAS wild-type status was predictive of the benefit of adjuvant chemotherapy in a sub-analyses performed in the JBR.10 trial (HR 0.69, 95% CI 0.49 to 0.97; P = 0.03) [18]. Patients with KRAS mutations derived no benefit from the use of adjuvant chemotherapy compared with observation (HR 0.95, 95% CI 0.53 to 1.71; P = 0.87). However, the P value for interaction was not significant. A similar trend was observed in the CALGB9633 for patients with wild-type KRAS allocated to adjuvant carboplatin–paclitaxel (HR 0.69) but again without statistical significance [25]. In a pooled analysis of KRAS mutations in the LACE-Bio project, the KRAS mutation status predicted no benefit from adjuvant chemotherapy [26]. In patients with advanced NSCLC, a large phase III trial has demonstrated overall survival benefit with the addition of cetuximab [27]. The value of KRAS status in NSCLC is not established, when compared with colorectal cancer patients, where the use of cetuximab is restricted to wild-type KRAS patients. In the BMS099 study, which compared platinum–taxane-based chemotherapy alone or plus cetuximab in advanced NSCLC, the presence of KRAS mutations was not associated with a lack of benefit from cetuximab [28, 29].

**EGFR**

**EGFR mutations.** The effect of EGFR mutation on patients with early-stage NSCLC receiving adjuvant chemotherapy has been reported from patients enrolled in the JBR.10 study. The presence of sensitizing mutations resulted in relatively greater benefit in patients receiving adjuvant chemotherapy (HR 0.44, 95% CI 0.11 to 1.70, P = 0.22) compared with wild-type patients (HR 0.78, 95% CI 0.58 to 1.06, P = 0.12), but this quantitative difference was not significant (interaction P = 0.50) [30].

**EGFR expression.** The effect of EGFR gene copy number on patients with early-stage NSCLC receiving adjuvant chemotherapy has also been studied in the JBR.10 study [30]. High EGFR copy was neither significantly prognostic nor predictive, although quantitatively it was associated with greater benefit from adjuvant chemotherapy. In the FLEX trial, the value of an EGFR IHC score as a predictive biomarker for the addition of cetuximab to chemotherapy has been analyzed [31]. For patients in the high EGFR expression group, overall survival was longer in the chemotherapy/cetuximab group than in the chemotherapy alone group (median 12.0 versus 9.6 months, HR 0.73, 0.58–0.93; P = 0.011), with no meaningful increase in side effects. There was no survival benefit for patients in the low EGFR expression group (median 9.8 months versus 10.3 months; HR 0.99, 0.84–1.16; P = 0.88).

**gene expression profiling**

The multigene expression technologies have the potential of interrogating thousand of genes simultaneously. Several different platforms and analysis tools are available what makes signatures and genes vary one from another and make reproducibility more difficult. A 15-gene signature as a predictor of benefit from adjuvant chemotherapy was defined from patients enrolled in the JBR.10 study [32]. This signature separated patients into high-risk and low-risk subgroup and correlated with survival. In the high-risk group, treatment with vinorelbine/cisplatin conferred significant survival benefit compared to observation alone (HR 0.33, 0.17–0.63; P = 0.0005) but low-risk patients did not (HR 3.67, 1.22–11.06, P = 0.01). Further validation studies are needed before implementation of theses signatures in clinical practice.

**microRNAs (miRNAs)**

miRNAs are short ribonucleic acid (RNA) molecules of 20 to 22 nucleotides involved in post-transcriptional regulation that bind to complementary sequences on target messenger RNA transcripts resulting in translational repression or target degradation and gene silencing, thus makes miRNAs be related to cell growth, differentiation and survival. In the IALT-Bio project expression of miR-21, miR-29b, miR-34a-b-c, miR-155 and let-7a were analyzed [33]. Expression of these miRNAs, alone or combined, was not predictive for benefit from adjuvant chemotherapy.

**conclusion**

Individualized therapy in patients with NSCLC on the basis of predictive biomarkers has the potential to improve outcomes. The identification of EGFR mutations and ALK translocations in NSCLC patients has defined particular populations with different treatment options. Whether a similar approach could be make for patients receiving chemotherapy remains unanswered. At present, several predictive biomarkers have been purposed and their validation in prospective trials is needed. Technical issues are relevant; several methods have been used and its standardization needs to be performed before its implementation in the clinic. Nevertheless, all these results are encouraging and results from ongoing and future trials will better define the useful of biomarkers to predict benefit derived from chemotherapy.

**disclosure**

The authors have declared no conflicts of interest.

**references**


