The Evolving Role of Molecular Markers in the Management of Non-Small Cell Lung Cancer

The Importance of Identifying Molecular Markers in Non-Small Cell Lung Cancer

To understand the importance of molecular markers in the current and future treatment of lung cancer, one should first understand how lung cancer was classified up until the beginning of this decade. Pathologists would look at a sample of a patient’s lung tumor under a microscope, and then make a judgment of whether the cells represented small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). Although that is an oversimplification, for all practical purposes, that is what oncologists cared about when it came to choosing treatment. If the diagnosis was NSCLC, then oncologists treated the patient with platinum doublet chemotherapy using one of many standard regimens that were felt to be equally effective. Unfortunately we knew that these regimens only worked in a certain proportion of patients, but we had no way to predict ahead of time who would benefit and who would not.

At the same time pathologists and molecular biologists have known for some time that NSCLC is not really just one disease, but rather a constellation of many diseases that all share the distinction of starting in the lung. For example, major subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma were often reported in pathology reports but did not influence treatment choice. Since 2004 we have taken this one step farther, asking pathologists to tell us not just that the lung cancer is non-small cell but also that it is non-squamous cell, for purposes of safety with Avastin (bevacizumab) and efficacy with Alimta (pemetrexed), but that is the topic for another chapter.

As our understanding of the molecular basis of cancer has grown, we have developed a number of new molecularly-targeted agents with promise in the treatment of lung cancer. However, targeted drugs tend to have limited or no effect on cancers that lack the “target” of the drug, creating a need for markers to guide us.

Molecular Markers

A molecular marker is an identifiable molecular characteristic (DNA, RNA, or protein) that can be used to provide prognostic or predictive information about the cancer. A prognostic marker is one which indicates a better or worse outcome irrespective of treatment. In contrast a predictive marker indicates a better or worse chance of an outcome for a specific treatment. This type of marker is the holy grail of translational research and a necessity for true “personalized medicine”, namely a way to tell ahead of time which treatment will or will not work.

In this chapter, I will discuss the evidence to date of a number of molecular markers in clinical development. The detailed description of these markers and how they are tested was covered in a separate chapter.
The Epidermal Growth Factor Receptor (EGFR)

In the early 2000s the first-generation EGFR tyrosine kinase inhibitors (TKIs) Iressa (gefitinib) and Tarceva (erlotinib) were tested in patients with non-small cell lung cancer. Based on the results from the randomized BR.21 trial, which showed an approximately 2 month improvement in overall survival for unselected NSCLC patients treated with Tarceva compared with placebo, Tarceva was approved for the second and third-line treatment of advanced NSCLC.

However, what was evident from these trials was that about 10% of Western patients treated with either of these drugs had dramatic and sometimes long-lasting responses. When they looked at the responders, they found that a large proportion were women, all had adenocarcinoma, many were of Asian ethnicity, and most had either never smoked or smoked very little compared to average NSCLC patients. In 2004, investigators at the Dana Farber Cancer Institute and at Massachusetts General Hospital in Boston, and also at Memorial Sloan Kettering Cancer Center in NYC, simultaneously published results showing that most of these “dramatic responders” had recurring mutations in the tyrosine kinase (TK) domain of the EGFR gene.

Since those first discoveries in 2004, a number of prospective trials have shown that the presence of an activating EGFR mutation in the tumor predicts a high likelihood of objective tumor response, up to 70-80%. In 2009, Dr. David Jackman at the Dana Farber Cancer Institute in Boston published the compiled results from all the prospective trials of Tarceva or Iressa in EGFR mutant NSCLC, comprising 223 patients. The overall response rate (ORR) to TKIs was 67%, with an average time to progression of 11.8 months and a median overall survival of 23.9 months (compared to the 12-14 months in most advanced NSCLC trials). What was not evident from these non-randomized data, however, was whether the EGFR mutations predicted an improved survival with TKIs or if the mutations simply carried an improved prognosis irrespective of TKI treatment. For all anyone knew, these patients might live just as long with chemotherapy.

The first trial to test this concept was called the Iressa Pan Asian Study (IPASS), which randomized 1217 never or light-smoking Asian patients with previous-untreated advanced lung adenocarcinoma to either Iressa or carboplatin and paclitaxel. The trial showed an improvement in progression-free survival (PFS) in the Iressa arm compared to chemotherapy, but more importantly 437 of these patients had tissue available for determination of EGFR mutation status. In patients with known EGFR mutation, there was a highly significant improvement in PFS compared to chemotherapy (curve B below). In patients who were EGFR wild-type, chemotherapy was superior for PFS (curve C).
Of note, there did not appear to be any difference in overall survival (OS) between the two arms, which may have been explained by patients crossing over to the other arm at the time of progression. Consistent with the Jackman data, *EGFR* mutant patients had a response rate to Iressa of 71% (versus 41% with chemo), while those who were *EGFR* wild-type had an ORR of 1.1% with Iressa versus 23% with chemo.

Of course, an even better idea would be to test TKIs versus chemotherapy in patients with proven *EGFR* mutations alone, which was done by Maemondo and colleagues and published in 2010. The investigators randomized 230 patients with EGFR mutant tumors to Iressa or carboplatin and paclitaxel, again showing a significant improvement in PFS with the TKI.
Similar to the IPASS trial, there was no difference in median OS between the groups, although 95% of patients who progressed on chemotherapy went on to receive Iressa second-line, which likely masked any survival advantage. The median OS was 30 months for patients on the Iressa arm and 23.6 months on the chemo arm.

Taken together, it is clear now that first-line treatment with an EGFR TKI, which in the USA means Tarceva, is very reasonable and should be a standard therapy in patients with proven EGFR mutations. On the other hand, the poor PFS in wild-type patients treated with Iressa indicates that **TKIs should not be used first-line in EGFR wild-type patients** even in never-smoking Asian women with adenocarcinoma. In the common situation where **EGFR mutation status in unknown, EGFR TKIs should not be used first-line empirically based on clinical characteristics**, but rather should be reserved for second line according to the approved indication. Finally, for people who worry that Iressa and Tarceva may not be equally effective in this population, there is a similar phase III trial of Tarceva versus chemotherapy in EGFR mutant NSCLC patients underway in Europe called the EURTAC trial.

**EGFR Gene Amplification by FISH**

I think this is worth a paragraph to discuss, since it continues to emerge as a question in trials. One of the potential biomarkers for benefit from EGFR inhibitors is amplification of the EGFR gene, as assessed by fluorescent in situ hybridization (FISH) ([see prior post for discussion](http://cancergrace.org/lung/2010/10/31/integration-of-molecular-markers-in-practice-for-nsclc-ref-lib-np/)). FISH positivity was first reported to be potentially predictive of benefit based on very limited tissue data (56 patients) from the randomized BR.21 trial comparing Tarceva to placebo in previously treated patients with advanced NSCLC.

However, one of the complicating factors in assessing the usefulness of FISH-positivity as a marker is the fact that almost all patients with EGFR mutations are also FISH+, and almost no trials showed both the FISH status and mutation status in the same patients—until IPASS, that is. Tissue analysis from IPASS showed that patients with FISH+ tumors, but were **EGFR wild-type, did not have more benefit from Iressa than from chemotherapy**. For now it
appears that **FISH testing for EGFR is not clearly valuable as a marker predicting benefit from TKIs independent of EGFR mutations.**

FISH positivity has also been proposed as a potential marker for benefit from the anti-EGFR antibody Erbitux (cetuximab). Cetuximab, when added to cisplatin and vinorelbine in advanced NSCLC patients in the phase III FLEX trial, significantly (although modestly) improved OS. In a smaller phase II study conducted by SWOG, Dr. Hirsch in Colorado showed that patients with FISH+ tumors benefited more from the addition of cetuximab to chemotherapy than those whose tumors were FISH- in both PFS and OS.

![SWOG 0342: PFS and OS by EGFR FISH Status](image)

However, data presented at the 2009 ASCO meeting from the two phase III cetuximab lung trials, **FLEX** and **BMS 099**, indicated that **FISH-positivity was not predictive of benefit from cetuximab**, and for now should not be used as a biomarker for this purpose.

**KRAS Mutations**

Mutations in the Kirsten rat sarcoma virus (K-RAS) gene are common in NSCLC and are frequently associated with tobacco smoke, as detailed in another chapter. **KRAS** mutations are mutually exclusive of EGFR mutations, and there is a common perception that the presence of an EGFR mutation confers resistance to EGFR TKIs like Tarceva and Iressa. This concept comes from retrospective data showing that patients with **KRAS** mutations have essentially no objective responses to TKIs.
However, it is unclear if tumors with \textit{KRAS} mutations derive no benefit from EGFR TKIs or if they simply derive the same modest benefit that all patients with \textit{EGFR} wild-type tumors derive. Again returning to Dr. Jackman’s database of outcomes in EGFR TKI trials, there is evidence that patients with \textit{KRAS} mutation do about as well as \textit{KRAS} wild-type patients if the \textit{EGFR} gene is also wild-type. This is almost invariably the case, since \textit{EGFR} and \textit{KRAS} mutations are almost completely mutually exclusive.

Prospective data also suggest a benefit of TKIs in \textit{KRAS} mutants. Molecular subgroup analysis from the randomized SATURN trial, which compared maintenance Tarceva to placebo in advanced NSCLC, showed that \textit{KRAS} mutant patients derived approximately the same benefit from the maintenance Tarceva as did \textit{KRAS} wild-type patients (although not significant, based on small numbers of patients).

(Adapted from Brugger, W. ASCO 2009)
So the conclusion based on the evidence to date is that **KRAS mutations should not preclude a patient from receiving Tarceva** either as maintenance therapy or as second or third-line treatment for NSCLC according to the FDA indication.

KRAS has also been examined as a potential marker for lack of benefit from cetuximab. In advanced colorectal carcinoma, **KRAS** mutations (although slightly different ones than seen in lung cancer) are strong **negative predictors** of benefit from cetuximab, and it was assumed this would also hold true in NSCLC. However, this turned out not to be the case.

Data from both the FLEX and BMS099 trial also showed that **KRAS** mutation status made no difference in outcome for NSCLC patients treated with cetuximab. Therefore, there does not appear to be any clear evidence at the present time that **KRAS** mutation testing should be used for clinical decision making in lung cancer outside of a clinical trial.

**Markers of DNA Repair (ERCC1 and RRM1)**

Traditional cytotoxic chemotherapy is the primary treatment for the majority of advanced NSCLC patients and will remain an important part of the lung cancer armamentarium for many years to come. Since any particular chemotherapy treatment only causes clinical benefit in a subset of patients, molecular markers that predict potential benefit from existing chemotherapy drugs have the potential to help more NSCLC patients than the sexiest of the new molecular markers detailed above.

The backbone of chemotherapy treatment for NSCLC is the platinum (cisplatin or carboplatin) doublet. Platinum causes DNA adducts that must be repaired if a cell is to survive, and the most important molecule in repairing damage from platinum is called **excision repair cross complementation group-1 (ERCC1)**. ERCC1 levels can be measured in a tumor sample, and in retrospective analyses of early stage and advanced NSCLC patients, may be useful in predicting benefit (or lack therof) from platinum chemotherapy.

A second molecular marker of chemosensitivity is **ribonucleotide reductase-M1 (RRM1)**. RRM1 is the regulatory subunit of an important enzyme (ribonucleotide reductase (RR) that is involved in production of the building blocks of DNA, and RR is inhibited by the chemotherapy drug **gemcitabine**.

In general, the concept is simple. Low levels of ERCC1 or RRM1 tend to predict increased sensitivity to platinum or gemcitabine respectively, while high levels predict resistance. The data for ERCC1 is most convincing in early stage disease, which was reviewed nicely by Dr. Wakelee in a separate chapter. In advanced disease, there is definitely an inverse association of ERCC1 and RRM1 levels with response rates from chemotherapy, but the impact on survival has been harder to prove.

Retrospective studies have been published both **supporting a survival benefit from platinum in patients with low ERCC1 levels versus patients with high levels**, and **failing to show such an association**. However, the proof in the pudding must come from prospective trials that tests
treatment based on ERCC1 and RRM1 levels. Two of these have been published, and at least one is still ongoing.

In 2007, Cobo and colleagues published a phase III trial that randomized advanced NSCLC to either standard cisplatin and docetaxel chemotherapy or to treatment based on determination of their ERCC1 RNA levels (termed the “genotypic arm”). Patients with low levels of ERCC1 received cis/doc while those with high ERCC1 received docetaxel and gemcitabine (a non-platinum doublet that is considered to be generally equivalent in efficacy to platinum-doublet chemotherapy). The results showed a significantly increased response rate in the genotypic arm (51% versus 39%), but the same overall survival of about 10 months in each arm.

More recently, Simon and colleagues designed a phase II trial testing the feasibility of designing treatment for advanced NSCLC patients based on ERCC1 and RRM1 RNA levels, termed the Molecular Analyses-Directed Individualized Therapy (MADeIT) trial. Patients were assigned to one of 4 different chemotherapy regimens with or without platinum and gemcitabine based on high or low levels of each marker (see below).

![Diagram of the MADeIT trial algorithm](image)

Enrolled patients did well overall, with an ORR of 44% and PFS and OS of 6.6 and 13.3 months, respectively. These numbers, however, are not unprecedented in a phase II trial, and a randomized trial is needed to confirm the benefit of this strategy. The phase III trial is called MADeIT II and is currently ongoing.

Why has it been so difficult to prove that ERCC1 and RRM1 levels predict for benefit (or lack thereof) from chemotherapy? The association with early stage NSCLC and adjuvant chemotherapy is fairly convincing, but metastatic disease is a much more complex situation. For example, it may matter what was biopsied to determine the marker levels. It has been shown that metastases and primary tumors express different levels of ERCC1 up to 41% of the time. Perhaps it is this heterogeneity that creates this problem; if so, perhaps a fresh biopsy from the metastatic site is needed to determine the treatment rather than using the primary
tumor. Perhaps the assay, which is performed and interpreted a little differently in different labs, is not reliable enough for routine use yet.

Regardless, this is a very promising area of research, and there is a good deal of attention being paid to developing these markers for treatment decision-making. However, a number of companies have jumped the gun and are already routinely testing for ERCC1, RRM1, and other potential molecular markers while trying to sell this as a standard of care today. I must emphasize that this is still experimental for now, and I do not recommend basing treatment decisions on these measurements until they have been sufficiently validated.

EML4-ALK translocation

The most exciting emerging molecular marker in patients with NSCLC is arguably the **EML4-ALK fusion gene**, which is present in approximately 4% of patients with NSCLC. *Early reports indicate it is most commonly seen in younger patients (50s), males, never smokers, and patients with adenocarcinoma*. This new oncogene also appears to be mutually exclusive of *EGFR* and *KRAS* mutations. The **EML4-ALK translocation** is a very exciting new target and potential predictive marker for benefit from drugs that inhibit ALK, which are still in early clinical development and are only available as part of a clinical trial. However, given the enormous potential benefit from these drugs, and the aggressive strategies being taken by the pharmaceutical companies, they may become available much relatively soon.

One of the nice things about the ALK fusion gene is that the diagnostic test is already available at many large hospitals. The **break-apart FISH assay** is already used to diagnose a rare form of lymphoma, although the interpretation in this setting is more complex.

Although this is still a new concept, there has been a very rapid (by historic standards) move by pharmaceutical companies to develop drugs to target ALK. The drug farthest in development is crizotinib (PF-02341066; Pfizer), which was originally developed to inhibit a molecular target called MET, but is also a very good ALK inhibitor. At the 2010 ESMO meeting in Europe, the most recent results from a phase I trial of crizotinib in 111 NSCLC patients with tumors harboring ALK translocations was presented. The overall response rate (ORR) was 57%, with 70% of patients still on treatment. The side effects appear to be mild thus far, with mild GI upset (nausea, diarrhea) and visual disturbances in light-dark accommodation being reported as the most common toxicities.

Based on these promising phase I results, a phase III registration trial for ALK+ NSCLC patients who have failed one prior chemotherapy regimen has been initiated. Patients will be randomized to either crizotinib or second-line chemotherapy with a primary endpoint of PFS. ALK+ patients who are either ineligible for the phase III trial or who progress on the chemotherapy-alone arm of the phase III trial may enroll on a single-arm phase II crizotinib trial.
Conclusions

Molecular markers are identifiable signs in patients or in tumor tissue that have the potential to provide information about prognosis or even to predict which treatments work best in an individual cancer patient, and form the backbone of personalized medicine. For now, only \textit{EGFR} mutations are sufficiently validated to be used in routine management of patients with NSCLC, but in my opinion ALK and ERCC1 are not far behind. Although much work remains to be done, the time is rapidly approaching when molecular markers will be routinely used to match every new or relapsing lung cancer patient with the most effective and least toxic treatment.

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