PROGNOSTIC AND PREDICTIVE BIOMARKERS IN NSCLC

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Prognostic versus predictive

- Prognostic: In presence of the biomarker patient outcome independent of the treatment
- Predictive: In presence of the biomarker patient outcome is different according to the treatment
### Predictive Factors for EGFR-TKI Sensitivity

#### Predictive for Response

- **Clinical**
  - Gender
  - Histology
  - Smoking history
  - Ethnicity

- **Biological**
  - EGFR Gene mutation
  - EGFR high copy number
  - HER2 high copy number
  - Akt activation

#### Predictive for Survival

- Smoking history
- Response to prior therapy
- PS
- Histology
- Previous Platinum
- Skin rash
- Ethnicity

#### Predictive for Resistance

- K-Ras Mutation
- EGFR exon 20 insertion
- HER2 exon 20 mutation
- EGFR T790M-D761Y
- MET Amplification

#### Primary Resistance

#### Acquired Resistance
**EGFR mutations in prospective studies: the strongest predictor for response**

<table>
<thead>
<tr>
<th>Reference</th>
<th>#</th>
<th>Selection criterion</th>
<th>Line</th>
<th>Drug</th>
<th>RR (%)</th>
<th>PFS (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asahina</td>
<td>16</td>
<td>EGFR mutation</td>
<td>I</td>
<td>Gefitinib</td>
<td>75</td>
<td>8.9</td>
<td>Not reached</td>
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<td>30</td>
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<td>EGFR mutation</td>
<td>I</td>
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<td>75</td>
<td>9.7</td>
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<td>Yang</td>
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<td>8</td>
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<td>Gefitinib</td>
<td>63.2</td>
<td>7.1</td>
<td>20</td>
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<td>Gefitinib</td>
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<td>12.9</td>
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<td>Gefitinib</td>
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<td>15.4</td>
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<td>Yoshida</td>
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<td>Gefitinib</td>
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<td>7.7</td>
<td>Not reached</td>
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<td>Han</td>
<td>17</td>
<td>EGFR mutation</td>
<td>I/II+</td>
<td>Gefitinib</td>
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<td>21.7</td>
<td>30.5</td>
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<td>Tamura</td>
<td>28</td>
<td>EGFR mutation</td>
<td>I/II/III</td>
<td>Gefitinib</td>
<td>75</td>
<td>11.5</td>
<td>Not reached</td>
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</table>
EGFR-TKIs versus chemotherapy in first-line: Phase III trials in “clinically selected” patients

**IPASS**
- Chemonaive
- Age > 18
- Adenocarcinoma
- Never/light smokers
- ECOG PS: 0-2
- Stage IIIB-IV

Gefitinib (250 mg / day)

**FIRST SIGNAL**
- Chemonaive
- Age 18-75 years
- Adenocarcinoma
- Never smokers
- ECOG PS: 0-2
- Stage IIIB-IV

Gefitinib (250 mg / day)

Carboplatin (AUC 5 or 6) / paclitaxel (200 mg / m²)
3 weekly#

Gemcitabine 1250 mg/mq 1,8
Cisplatin 80 mg/mq 1
Q 21 days, up to 9 cycles

Primary end-point: PFS
Gefitinib demonstrated superiority relative to carboplatin / paclitaxel in terms of PFS.

Primary Cox analysis with covariates
HR <1 implies a lower risk of progression on gefitinib
Progression-free Survival in EGFR Mutation Positive and Negative Patients

**EGFR mutation positive**

Gefitinib (n=132)
Carboplatin / paclitaxel (n=129)

HR (95% CI) = 0.48 (0.36, 0.64)

p<0.0001

No. events gefitinib, 97 (73.5%)
No. events C / P, 111 (86.0%)

ITT population
Cox analysis with covariates

**EGFR mutation negative**

Gefitinib (n=91)
Carboplatin / paclitaxel (n=85)

HR (95% CI) = 2.85 (2.05, 3.98)

p<0.0001

No. events gefitinib, 88 (96.7%)
No. events C / P, 70 (82.4%)

Treatment by subgroup interaction test, p<0.0001
EGFR-TKIs versus chemotherapy in first-line: Phase III trials in “biologically selected” patients

**NEJ002**
- Chemonaive
- Age 20-75 years
- EGFR mutation+
- ECOG PS:0-1
- Stage IIIB-IV
- Gefitinib (250 mg / day)
- Carboplatin/ paclitaxel q 3 weeks

**WJTOG3405**
- Chemonaive
- Age >20 years
- EGFR Mutation+
- ECOG PS:0-1
- Stage IIIB-IV
- Gefitinib (250 mg / day)
- docetaxel 60 mg/mq
- Cisplatin 80 mg/mq
- Q 21 days, up to 6 cycles

Primary end-point: PFS
Gefitinib more effective than chemotherapy in EGFR Mutation+ NSCLC

NEJ002: PFS

WJTOG3405

<table>
<thead>
<tr>
<th></th>
<th>Gef</th>
<th>CT</th>
<th>p</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (%)</td>
<td>56.3</td>
<td>25.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS (months)</td>
<td>9.2</td>
<td>6.3</td>
<td>&lt;0.001</td>
<td>0.48</td>
</tr>
</tbody>
</table>

HR 0.36 95% CI 0.25, 0.51
p<0.001
Median 10.4 vs 5.5 months
SATURN study design

Stratification factors:
- EGFR IHC (positive vs negative vs indeterminate)
- Stage (IIIB vs IV)
- ECOG PS (0 vs 1)
- CT regimen (cis/gem vs carbo/doc vs others)
- Smoking history (current vs former vs never)
- Region

Co-primary endpoints:
- PFS in all patients
- PFS in patients with EGFR IHC+ tumors

Secondary endpoints:
- OS in all patients and those with EGFR IHC+ tumors, OS and PFS in EGFR IHC– tumors; biomarker analyses; safety; time to symptom progression; QoL

*Cisplatin/paclitaxel; cisplatin/gemcitabine; cisplatin/docetaxel cisplatin/vinorelbine; carboplatin/gemcitabine; carboplatin/docetaxel carboplatin/paclitaxel
Largest PFS benefit with erlotinib in patients with *EGFR* mutated tumours

**EGFR mutation+**

HR=0.10 (0.04–0.25)  
Log-rank p<0.0001  
Erlotinib (n=22)  
Placebo (n=27)

**EGFR wild-type**

HR=0.78 (0.63–0.96)  
Log-rank p=0.0185  
Erlotinib (n=199)  
Placebo (n=189)

Interaction p<0.001
ATLAS Study Design

Eligibility
- Stage III/IV NSCLC
- ECOG performance status 0-1

Stratification factors
- Gender
- Smoking history (never vs former/current)
- ECOG performance status (0 v $\geq$1)
- Chemotherapy regimen

Carbo/paclitaxel; cis/vinorelbine; carbo or cis/gemcitabine; carbo or cis/docetaxel.

Primary endpoint
- PFS in all randomized pts

Secondary endpoints
- Overall survival
- Safety

Exploratory endpoints
- Biomarker analyses (IHC, FISH, EGFR & K-Ras mutation)
PFS K-M Curves by *EGFR* Mutation Status

**EGFR Wild-Type**
- HR = 0.850
  - 95% CI: 0.638 - 1.131
  - Log-rank *P*=0.2620

**EGFR Mutant**
- HR = 0.439
  - 95% CI: 0.223 - 0.864
  - Log-rank *P*=0.0137

No. of patients at Risk
- B+E: 150 66 25 12 4 0 0 0 27 19 12 6 3 1 1 1 0
- B+P: 145 58 27 11 3 1 0 0 25 10 3 2 2 1 1 1 0
IS EGFR MUTATION TESTING THE BEST PREDICTOR FOR PATIENT SURVIVAL?
EGFR Mutations: A Positive Prognostic Factor?

TRIBUTE

INTACT 1&2
No trial demonstrated survival benefit for EGFR mutated patients treated with TKIs.

**IPASS**
- Gefitinib (n=132)
- Carboplatin / paclitaxel (n=129)
- HR (95% CI) = 0.776 (0.500, 1.202)
- No. events gefitinib, 38 (28.8%)
- No. events C / P, 43 (33.3%)

**SATURN**
- HR=0.83 (0.34–2.02)
- Log-rank p=0.6810

**First-SIGNAL**
- HR (95%CI) = 0.623 (0.352 to 1.022)
- P= 0.648 (by log rank test)
BR21: Survival According to Updated EGFR Mutation Status

A Exon 19 Deletions and L858R Mutations

- P = 0.12
- Hazard ratio, 0.55 (95% CI, 0.25-1.19)

B Wild-Type EGFR and Indeterminate Variants

- P = 0.09
- Hazard ratio, 0.74 (95% CI, 0.52-1.05)

Interaction P value = 0.47

Shepherd et al, ASCO 2007
# EGFR Gene Gain: A Prognostic Factor?

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Total Number</th>
<th>Survival (months)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Hirsch</td>
<td>FISH</td>
<td>183</td>
<td>15.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Jeon</td>
<td>FISH</td>
<td>262</td>
<td>44</td>
<td>NR</td>
</tr>
<tr>
<td>Suzuki</td>
<td>FISH</td>
<td>71</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NR: Not Reached; NA: Not available
EGFR Gene Copy Number and Survival in the NSCLC Cohort

Median survival:
- **EGFR FISH-**: 48.3 months
- **EGFR FISH HP**: 40.7 months
- **EGFR FISH GA**: 30.7 months

At risk: 376, 191, 4
Negative: 215, 111, 1
HP: 122, 61, 2
GA: 39, 19, 1

At risk: 376, 191, 4
FISH+: 161, 80, 3
FISH-: 215, 111, 1

Cappuzzo et al. JCO 2009
FISH Predicts Benefit of EGFR-TKIs

**ISEL FISH +**
- Log-rank: p=0.008
- HR=0.44 (0.23, 0.82)
- Cox: p=0.07
- HR=0.61 (0.36, 1.04)

**BR21 FISH +**
- Log-rank: p=0.008
- HR=0.44 (0.23, 0.82)

**ISEL FISH -**
- Cox: p=0.42
- HR=1.16 (0.81, 1.64)

**BR21 FISH -**
- Log-rank: p=0.59
- HR=0.85 (0.48, 1.51)

Hirsch 2005, Tsao 2005
EGFR EXPRESSION: THE WEAKEST PREDICTOR
EGFR IHC: No Prognostic Effect in Resected NSCLC in Large Meta-Analysis

Nakamura et al., Thorax 2005
# RESPONSE ACCORDING TO EGFR IHC - ISEL, IDEAL & BR.21

<table>
<thead>
<tr>
<th>EGFR Status</th>
<th>ISEL</th>
<th>IDEAL</th>
<th>BR.21</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ORR (%)</td>
<td>ORR (%)</td>
<td>ORR (%)</td>
<td>ORR (%)</td>
</tr>
<tr>
<td>EGFR +ve</td>
<td>N=158</td>
<td>N=84</td>
<td>N=106</td>
<td>N=348</td>
</tr>
<tr>
<td></td>
<td>13 (8.2%)</td>
<td>13 (13.4%)</td>
<td>12 (11.3%)</td>
<td>38 (10.9%)</td>
</tr>
<tr>
<td>EGFR -ve</td>
<td>N=69</td>
<td>N=17</td>
<td>N=80</td>
<td>N=166</td>
</tr>
<tr>
<td></td>
<td>1 (1.5%)</td>
<td>1 (5.6%)</td>
<td>3 (3.8%)</td>
<td>5 (3.0%)</td>
</tr>
</tbody>
</table>

*P=0.003
BR.21 Survival According to EGFR Protein Expression

**HER1/EGFR+**

Log-rank: p=0.02  
HR=0.68 (0.49, 0.95)

**HER1/EGFR−**

Log-rank: p=0.70  
HR=0.93 (0.63, 1.36)

p value for interaction = 0.25

SATURN: PFS in EGFR IHC+ tumors

HR = 0.69 (0.58–0.82)  HR: 0.71 in the whole population
Log-rank p < 0.0001

<table>
<thead>
<tr>
<th></th>
<th>Erlotinib</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>PFS at 12 wks (%)</td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td>PFS at 24 wks (%)</td>
<td>32</td>
<td>18</td>
</tr>
</tbody>
</table>

PFS probability

Erlotinib (n=307)
Placebo (n=311)

PFS is measured from time of randomization into the maintenance phase; assessments were every 6 weeks
OTHER BIOMARKERS: KRAS AND MET
KRAS Mutations and Survival: Prognostic or Predictive?

- Over 50 studies published
- Different methods for detection (IHC versus PCR)
- Conflicting results

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>% Mutated</th>
<th>p value</th>
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<tbody>
<tr>
<td>Tsao</td>
<td>450</td>
<td>26.0</td>
<td>0.3</td>
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<tr>
<td>Schiller</td>
<td>197</td>
<td>24.0</td>
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<td>Graziano</td>
<td>260</td>
<td>16.4</td>
<td>0.3</td>
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<td>Siegfried</td>
<td>181</td>
<td>31.5</td>
<td>0.6</td>
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<td>Fukuyama</td>
<td>159</td>
<td>6.9</td>
<td>&lt;0.05</td>
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<tr>
<td>Huang</td>
<td>144</td>
<td>8.3</td>
<td>0.03</td>
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<td>Miyake</td>
<td>187</td>
<td>8.0</td>
<td>0.03</td>
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</table>
BR.21: prognostic analysis for KRAS mutation (PFS) in placebo arm

Log-rank p=0.917

Conclusion: not prognostic
SATURN: prognostic analysis for KRAS mutation (PFS) in placebo arm

Log-rank $p=0.0169$

Conclusion: prognostic
ATLAS: prognostic analysis for KRAS mutation (PFS) in placebo arm

![Graph showing survival probability over time for different groups]

- Placebo (KRAS MUT+) n=46
- Placebo (KRAS WT) n=115

Log rank p=0.03564

Conclusion: prognostic
KRAS Mutations: predictive for worst survival?

BR21

KRAS Wild Type

Median: 7.5 (5.4, 10.7)     3.4 (3.0, 7.1)
HR=0.69 (0.49, 0.97)   p=0.0311

KRAS Mutation

Median: 3.7 (1.9, 7.9)     7.0 (1.7, 19.5)
HR=1.67 (0.62, 4.50)   p=0.3096

TRIBUTE

• Few data in low patient number
• ~50% of KRAS mutated are EGFR FISH+

BR21 TRIBUTE
SATURN: PFS according to KRAS status

KRAS MUT+

HR = 0.75 (0.49–1.15)
Log-rank p = 0.2246

KRAS WT

HR = 0.73 (0.60–0.90)
Log-rank p = 0.0009

Interaction p = 0.95

Tarceva (n=49)
Placebo (n=41)

Tarceva (n=205)
Placebo (n=198)
OS in SATURN: biomarker subgroup analyses

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>HR (95% CI)</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>All</td>
<td>0.81 (0.70–0.95)</td>
<td>889</td>
</tr>
<tr>
<td>EGFR IHC+</td>
<td>0.77 (0.64–0.93)</td>
<td>621</td>
</tr>
<tr>
<td>EGFR IHC-</td>
<td>0.91 (0.59–1.38)</td>
<td>121</td>
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<tr>
<td>EGFR FISH+</td>
<td>0.96 (0.71–1.30)</td>
<td>232</td>
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<tr>
<td>EGFR FISH-</td>
<td>0.77 (0.58–1.03)</td>
<td>256</td>
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<td>KRAS mutation+</td>
<td>0.79 (0.49–1.27)</td>
<td>90</td>
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<tr>
<td>KRAS wild-type</td>
<td>0.86 (0.68–1.08)</td>
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<tr>
<td>EGFR mutation+</td>
<td>0.83 (0.34–2.02)</td>
<td>49</td>
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<tr>
<td>EGFR wild-type</td>
<td>0.77 (0.61–0.97)</td>
<td>388</td>
</tr>
</tbody>
</table>
MET FISH Results

Total evaluated: 435

- Low copy number: 383 (88.9%)
- High polysomy: 30 (7.0%)
- Gene amplification: 18 (4.1%)
Survival of Resected NSCLC According to MET Copy Number

- MET <5 copies/cell (N=383)
- MET ≥5 copies/cell (N=48)

**Median survival:**
- MET FISH-: 47.5 months
- MET FISH+: 25.8 months

**At risk**

- <2 copies/cell: 431
- ≥2 - <3 copies/cell: 129
- ≥3 - <4 copies/cell: 149
- ≥4 - <5 copies/cell: 95
- ≥5 - <6 copies/cell: 28
- ≥6 copies/cell: 20

**Survival data**

- At risk: 431
- MET-: 200
- MET+: 216

**P-value:** 0.0045

Cappuzzo et al., JCO 2009
Conclusions

• EGFR expression is the weakest predictor with no prognostic role
• At the gene level EGFR testing identifies patients with the highest benefit in response (mutation) or survival (FISH)
• KRAS testing is not recommended in clinical practice for patient selection
• MET gene copy number is a negative prognostic factor