Comprehensive Analysis of Serum Biomarkers and Tumor Gene Mutations Associated With Clinical Outcomes in the Phase 3 Study of (E7080) LEnvatinib in Differentiated Cancer of the Thyroid (SELECT)

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Background

• Lenvatinib is an oral, multikinase inhibitor of the VEGFR1–3, FGFR1–4, PDGFRα, RET, and KIT signaling pathways:
  – In the phase 3 Study of (E7080) LEnvatinib in Differentiated Cancer of the Thyroid (SELECT) for the treatment of RR-DTC, lenvatinib significantly prolonged median PFS by 14.7 months compared with placebo (HR 0.21; 99% CI, 0.14–0.31).

• To date, there are no established prognostic or predictive biomarkers for RR-DTC or its treatments:
  – Exploratory biomarker analyses in phase 2 trials of lenvatinib in RR-DTC have identified correlations between baseline Ang2 levels and genetic alterations in tumors (RAS/RAF mutations) with patient outcome
  – Ang2 regulates angiogenesis through Tie2.

• We present the results of the biomarker analyses of the placebo-controlled phase 3 SELECT trial.
### Patient Demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ITT Population (N = 392)</th>
<th>Serum Biomarker Analysis Population (n = 387)</th>
<th>Tumor Gene Mutation Analysis Population (n = 183)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range)</td>
<td>61.9 (21, 89)</td>
<td>61.9 (21, 89)</td>
<td>61.3 (21, 85)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>192 (49)</td>
<td>189 (49)</td>
<td>80 (44)</td>
</tr>
<tr>
<td>ECOG Performance Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>377 (96)</td>
<td>372 (96)</td>
<td>178 (97)</td>
</tr>
<tr>
<td>2–3</td>
<td>15 (4)</td>
<td>15 (4)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular, all</td>
<td>133 (33.9)</td>
<td>132 (34.1)</td>
<td>60 (32.8)</td>
</tr>
<tr>
<td>Hürthle cell</td>
<td>58 (14.8)</td>
<td>58 (15)</td>
<td>25 (13.7)</td>
</tr>
<tr>
<td>Papillary, all</td>
<td>259 (66.1)</td>
<td>255 (65.9)</td>
<td>123 (67.2)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>47 (12)</td>
<td>47 (12.1)</td>
<td>19 (10.4)</td>
</tr>
<tr>
<td>PFS HR* (95% CI) P-value</td>
<td>0.20 (0.15–0.27)</td>
<td>0.20 (0.15–0.26)</td>
<td>0.19 (0.12–0.28)</td>
</tr>
</tbody>
</table>

*Analyses are not stratified.

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CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; ITT, intent-to-treat; PFS, progression-free survival.
Analysis Groups and PFS

**Overall**

*Placebo vs Lenvatinib*  
HR = 0.20 (95% CI 0.15–0.27), *P* < 0.001  
3.6 mo (2.2–3.7) vs 18.3 mo (15.1–NA)

**Serum biomarker**

*Placebo vs Lenvatinib*  
HR = 0.20 (95% CI 0.15–0.26), *P* < 0.001  
3.6 mo (2.2–3.7) vs 18.7 mo (15.1–NA)

**Tumor gene mutation**

*Placebo vs Lenvatinib*  
HR = 0.19 (0.12–0.28), *P* < 0.001  
2.8 mo (1.9–3.7) vs NA (14.8–NA)

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**PFS HRs (95% CI) in all groups were similar.**

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26–30 September 2014, Madrid, Spain  
mo, months; NA, not available.
TUMOR MUTATIONS/GENETIC BIOMARKERS
Genetic Biomarker Analysis in SELECT

Archival tumor tissues were obtained from 220 patients.
183 Samples were analyzed by amplicon sequencing by Ion Torrent PGM for:
- **BRAF**: V600
- **NRAS/KRAS/HRAS**: G12, G13, Q61
- Mutation call criteria: > 500x coverage, > 5% frequency.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cohort</th>
<th>All</th>
<th>Tumor Gene Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>NA</td>
</tr>
<tr>
<td><strong>RAS</strong></td>
<td>All</td>
<td>392</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>Lenvatinib</td>
<td>261</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>131</td>
<td>71</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>All</td>
<td>392</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>Lenvatinib</td>
<td>261</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>131</td>
<td>72</td>
</tr>
</tbody>
</table>

*1 Sample had no call.

MU, mutant; WT, wild type.
In this placebo-controlled analysis, lenvatinib PFS benefit vs placebo was maintained regardless of BRAF or RAS mutation status.

**Events/N**

<table>
<thead>
<tr>
<th></th>
<th>Lenvatinib</th>
<th>Placebo</th>
<th>HR (95% CI)</th>
<th>Median (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>48/123</td>
<td>53/60</td>
<td>0.19 (0.12, 0.28)</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (PTC + FTC) WT</td>
<td>41/97</td>
<td>36/40</td>
<td>0.15 (0.09, 0.24)</td>
<td>18.3</td>
</tr>
<tr>
<td>All (PTC + FTC) MU</td>
<td>7/26</td>
<td>16/19</td>
<td>0.17 (0.07, 0.41)</td>
<td>NE</td>
</tr>
<tr>
<td>PTC WT</td>
<td>27/53</td>
<td>24/25</td>
<td>0.21 (0.12, 0.38)</td>
<td>12.9</td>
</tr>
<tr>
<td>PTC MU</td>
<td>7/25</td>
<td>16/19</td>
<td>0.18 (0.07, 0.43)</td>
<td>NE</td>
</tr>
<tr>
<td>FTC WT</td>
<td>14/44</td>
<td>12/15</td>
<td>0.06 (0.02, 0.17)</td>
<td>NE</td>
</tr>
<tr>
<td>FTC MU</td>
<td>0/1</td>
<td>0/0</td>
<td>NE (NE, NE)</td>
<td>NE</td>
</tr>
<tr>
<td><strong>NRAS or KRAS or HRAS (RAS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (PTC + FTC) WT</td>
<td>36/88</td>
<td>46/53</td>
<td>0.20 (0.13, 0.32)</td>
<td>18.3</td>
</tr>
<tr>
<td>All (PTC + FTC) MU</td>
<td>12/34</td>
<td>7/7</td>
<td>0.12 (0.04, 0.36)</td>
<td>NE</td>
</tr>
<tr>
<td>PTC WT</td>
<td>26/54</td>
<td>39/43</td>
<td>0.27 (0.16, 0.46)</td>
<td>14.8</td>
</tr>
<tr>
<td>PTC MU</td>
<td>8/23</td>
<td>2/2</td>
<td>0.17 (0.03, 0.87)</td>
<td>NE</td>
</tr>
<tr>
<td>FTC WT</td>
<td>10/34</td>
<td>7/10</td>
<td>0.04 (&lt;0.01, 0.19)</td>
<td>NE</td>
</tr>
<tr>
<td>FTC MU</td>
<td>4/11</td>
<td>5/5</td>
<td>0.05 (&lt;0.01, 0.45)</td>
<td>NE</td>
</tr>
</tbody>
</table>

FTC, follicular thyroid carcinoma; NE, not evaluable; PTC, papillary thyroid carcinoma.
PTC Patients With $BRAF^{WT}$ May Develop Rapidly Progressive Disease

- Lenvatinib PFS benefit vs placebo was maintained regardless of RAS/RAF mutation status:
  - Treatment * biomarker (response) interaction $P = 0.844/0.874$.
- $BRAF$ mutation may be a prognostic factor for PFS in progressive metastatic PTC:
  - Univariate (placebo): $BRAF^{WT}$ vs $BRAF^{MU}$: HR 0.48 (95% CI 0.25–0.92); Cox PH $P = 0.027$
  - Significance was maintained in a multivariate (placebo) analysis.
CIRCULATING SERUM BIOMARKERS
Serum Biomarker Analysis in SELECT

- Samples were collected at baseline, Cycle 1/Day 15, Day 1 of all subsequent treatment cycles until PD, and OT.
- Baseline serum samples were collected from 387 patients (98.7% of all randomized patients).
- Circulating CAFs were examined by ELISA:
  - VEGF, Ang2, soluble Tie2, TG

- Baseline Ang2 levels were correlated with MTS and ORR in the lenvatinib arm.
- Baseline Ang2 was a predictive factor for MTS:
  - Treatment * biomarker (response) interaction $P = 0.016$.  

**Correlation of baseline Ang2 with MTS**

Spearman Rank Correlation Test
- Lenvatinib (n = 239): $R = 0.31$, $P < 0.0001$
- Placebo (n = 124): $R = 0.16$, $P = 0.067$
### PFS Analysis: Dichotomized Subgroups of Baseline Serum Biomarker Levels

- Lenvatinib PFS benefit vs placebo was maintained regardless of baseline serum biomarker levels.
- Biomarkers were dichotomized into low (1st quartile) and high (all other quartiles) groups:
  - Kaplan-Meier curves of baseline Ang2 quartiles showed high PFS ratio (about 0.8 at 18 months) of the 1st quartile of the lenvatinib arm.
- HR in the low-baseline Ang2 subgroup (≤ 2556.06 pg/mL) was 3-fold lower than in the high subgroup.
- HR in the high Tg subgroup (> 159.5 ng/mL) was 2-fold lower than in the low subgroup.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Events/N</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>15/68</td>
<td>23/30</td>
</tr>
<tr>
<td>High</td>
<td>92/193</td>
<td>90/101</td>
</tr>
<tr>
<td>Baseline Ang2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>13/68</td>
<td>24/28</td>
</tr>
<tr>
<td>High</td>
<td>88/185</td>
<td>86/100</td>
</tr>
<tr>
<td>Baseline VEGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>26/62</td>
<td>26/33</td>
</tr>
<tr>
<td>High</td>
<td>76/190</td>
<td>82/93</td>
</tr>
<tr>
<td>Baseline Tie2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>25/61</td>
<td>30/36</td>
</tr>
<tr>
<td>High</td>
<td>79/196</td>
<td>82/94</td>
</tr>
<tr>
<td>Baseline TG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>24/58</td>
<td>24/30</td>
</tr>
<tr>
<td>High</td>
<td>71/180</td>
<td>74/84</td>
</tr>
</tbody>
</table>
Baseline Biomarker Levels and Clinical Outcomes

PFS by baseline Ang2 levels

- Low baseline Ang2 predicted PFS benefit from lenvatinib:
  - Univariate (lenvatinib arm): Low vs high Ang2 HR 3.40 (95% CI 1.90–6.10); Cox PH $P < 0.001$
  - Significance was maintained in multivariate (lenvatinib arm) analysis
  - Treatment*biomarker (response) interaction $P = 0.018$.

- High baseline TG levels may be a prognostic factor for PFS:
  - Univariate (placebo arm): Low vs high TG HR 1.71 (95% CI 1.06-2.75); Cox PH $P = 0.027$. 

PFS by baseline TG levels

- Treatment: $P < 0.001$
- Biomarker: $P = 0.023$
- Interaction: $P = 0.054$.
• Mean serum TG levels rapidly decreased with lenvatinib treatment (by C1D15), and remained low during lenvatinib treatment.
• Mean serum TG levels increased from baseline in the placebo arm.
• Decreased levels of TG were associated with lenvatinib response (C1D15 and later).
Baseline Thyroglobulin and Tumor Mutations

**BRAF status**

- MUT and NRAS have significantly low and high baseline TG levels, respectively, vs WT.

- $P < 0.0001$

**NRAS status**

- $P < 0.0344$

- MUT and NRAS have significantly low and high baseline TG levels, respectively, vs WT.
Conclusions

• Lenvatinib PFS benefit compared with placebo was maintained regardless of baseline circulating serum biomarker levels or BRAF/RAS mutational status.

• $BRAF^{V600}$ may be a positive prognostic factor in PTC:
  – PTC patients with $BRAF^{WT}$ may develop rapid disease progression.

• Baseline Ang2 levels were predictive for tumor size reduction and PFS in a subset of patients (lowest quartile, 0% to 25%) with lenvatinib treatment:
  – Ang2 may play a predictive role in defining sensitivity to lenvatinib.
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