PCSK9 is a member of the proteinase K subfamily of subtilisin-related serine endoproteases. The primary function of PCSK9 is to mediate the degradation of low-density lipoprotein receptor (LDLR) (1). PCSK9 expression is up-regulated by sterol regulatory element binding proteins. This family of transcription factors is responsible for the upregulation of the LDLr gene and other genes involved in cholesterol and fatty acid metabolism. Genetic analyses have shown that humans who have nonsense or loss-of-function mutations in the PCSK9 gene have lower plasma LDL cholesterol levels (2). In addition, increasing circulating levels of the protein are associated with increases in total cholesterol and LDL-C, but not HDL-C or triglyceride (3,4).

The current Pacific Biomarkers (PacBio) assay for PCSK9 is an ELISA that accurately quantifies recombinant factor or natural PCSK9 equally. This assay employs a sandwich enzyme immunoassay technique with a monoclonal PSCK9-specific immobilized capture antibody. An enzyme-linked polyclonal antibody specific for PCSK9 completes the sandwich. A PCSK9 assay based on AlphaLISA technology is also available for validation at Pacific Biomarkers. Potential advantages of the latter are improved sensitivity, analytical measurement range, and precision. In addition, PacBio has extensive experience in transferring proprietary assay into our laboratory for clinical trial implementation. PacBio will also provide appropriate consultation to identify the best assay available for a specific drug, and if needed, design and explore drug-interferences on the PCSK9 measurement.

Recently, the underlying mechanisms of PCSK9 action have been uncovered (see figure). Under normal physiologic conditions, PCSK9 is synthesized as a 74 kDa proprotein, which is autolytically cleaved to a 14 prosegment and a 60 kDa active subunit. The two subunits are tightly associated and are secreted together. Before secretion, some activated PCSK9 is processed at the N-terminal of the active protein by the enzyme furin. This action cleaves a 7 kDa peptide leaving a 53 kDa inactive PCSK9 (5).

The active form of PCSK9 can bind the LDLr, leading to internalization of the resulting receptor-PCSK9 complex to endosomes. Alternatively, LDLr binds to LDL and this complex is likewise directed to the endosome. The LDLr may be transported back to the cell membrane, while subsequent endosome-lysosome fusion leads to degradation of LDLr-PCSK9.

As described above an ELISA for circulating levels of PCSK9 is available at Pacific Biomarkers. This assay is designed in a way that both the 53 kDa and 60 kDa forms of PCSK9 are quantified. In addition, the assay recognizes PCSK9 that is bound to soluble LDLr. Thus, this method is considered a total PCSK9 assay.

The existing research clearly indicates that PCSK9 plays a key role in reducing hepatic LDLr levels. As such, PCSK9 regulates the amounts of LDLr and hence, modulates cholesterol levels by a mechanism that is independent of those targeted in earlier development of cholesterol-modifying drugs. Hence, PCSK9 has become an attractive drug target. It has been shown that fenofibrate produces a slight, but significant, decrease in serum PCSK9 (4), but preliminary data indicate antibody-based anti-PCSK9 drugs produce more substantial effects. Very recently three new phase 2 studies were published showing LDL-C reductions of about 40% to 60% compared to baseline (6, 7, 8, 9), similar to the findings reported earlier on REGN727 (10). More than half a dozen similar PCSK9 drugs are currently in development in stages ranging from preclinical to the start of Phase III (please see following Table and Reference 11 for complete overview). Another drug candidate is a biologic construct based on a fibronectin protein-binding domain. RNA inhibition is the basis for other drug entities and it has also been reported that small molecules that reduce PCSK9 are under development (11, 12). These drugs will meet a current unmet need for patients with familial hypercholesterolemia and patients that are resistant to statin treatment.
<table>
<thead>
<tr>
<th>Antibody-Based</th>
<th>Other High-Affinity Biologics</th>
<th>Expression Inhibition-Based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanofi/Regeneron</td>
<td>Bristol-Myers Squibb</td>
<td>Alnylam/Novartis</td>
</tr>
<tr>
<td>Amgen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfizer</td>
<td></td>
<td>Santaris/Novartis</td>
</tr>
<tr>
<td>Novartis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merck</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alder</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PCSK9 “Life” Cycle**

- **ER**
  - proPCSK9
  - LDLr

- **Golgi**
  - Furin
  - proPCSK9

- **Lysosome**
  - LDLr

- **Endosome**
  - LDL

- **Active + Inactive PCSK9 (“Total”)**
- **Free + Bound PCSK9 (“Total”)**

© 2012 Pacific Biomarkers


