

# Periostin and Dipeptidyl Peptidase-4

## Potential Biomarkers of Interleukin 13 Pathway Activation in Asthma and Allergy



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### KEYWORDS

• Asthma • Allergy • Biomarker • DPP-4 • IL-13 • Periostin

### KEY POINTS

- Periostin and dipeptidyl peptidase-4 (DPP-4) are both induced by interleukin (IL)-13 and can be measured in serum, making them potential biomarkers to guide anti-IL-13-directed therapy.
- Both proteins show increased expression in asthma and other diseases with type 2 inflammation, including atopic dermatitis (AD) and chronic rhinosinusitis with nasal polyps.
- Both proteins also have confounders to their expression, including age, induction by pro-fibrotic factors, body mass index (BMI), association with malignancies, and use of corticosteroids, which may have an impact on their specificity for type 2-driven disease.
- They demonstrate differences in their relationship with other type 2 biomarkers and no correlation with each other in serum, indicating they may be related to different aspects of IL-13-directed biology.
- Both have been used in clinical trials with anti-IL-13 monoclonal antibodies in severe asthma demonstrating enhanced efficacy in biomarker high subgroups; DPP-4 has also shown similar potential in a clinical trial in AD indicating their potential as biomarkers of IL-13-driven diseases.

### INTRODUCTION

IL-13 has been identified as a potential central effector cytokine in asthma and may contribute to many key pathologic features, including airway hyper-responsiveness, mucus production, airway remodeling, and structural changes, promoting B-cell proliferation and inducing class switching to immunoglobulin (Ig)G4 and IgE as well as promoting eosinophil survival, activation, and recruitment.<sup>1</sup> Although production of

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IL-13 can be inhibited by inhaled corticosteroids (ICS), there is a proportion of uncontrolled asthma patients who, despite treatment with high-dosage ICS and systemic corticosteroids, have elevated levels of IL-13 in sputum.<sup>2</sup>

Hence, the IL-13 pathway has been identified as a potential therapeutic target in severe asthma and allergic disease with selective monoclonal antibody therapies. Because there is heterogeneity of molecular mechanisms in asthma, it was hypothesized that to identify asthma patients with evidence of IL-13 pathway activation, a biomarker to direct anti-IL-13 therapy may be necessary.

Although it is possible to measure IL-13 levels in sputum, sputum induction and processing is a specialized technique and is not feasible in most clinical practice situations. Therefore, a blood-based sampling system is preferable. IL-13 concentrations in serum and plasma are generally below the level of reliable detection of most IL-13 assays (although more sensitive methods have been reported<sup>3</sup>); therefore, a surrogate of IL-13 pathway activation in the airway that is measurable in blood is required. This review evaluates the potential of 2 candidates of IL-13 pathway activation—serum periostin and dipeptidyl peptidase-4 (DPP-4)—for identifying asthma and allergic patients who may gain benefit from anti-IL-13-directed therapy (Fig. 1).

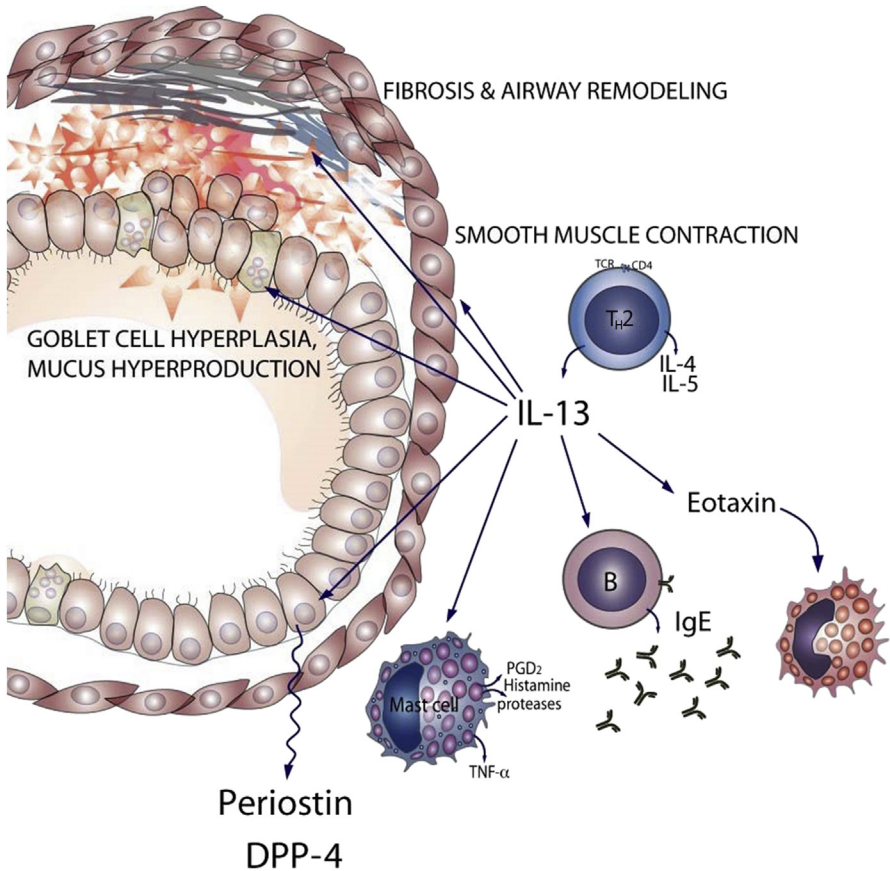


**Fig. 1.** IL-13 and its role in the pathology of T2 disease and in periostin and DPP-4 induction. (Modified from Brightling CE, She D, Ranade K, et al. Efficacy and safety of tralokinumab, an anti-IL-13 monoclonal antibody, in a phase 2b study of uncontrolled severe asthma. *Am J Respir Crit Care Med* 2014;189:A6670; with permission.)

## BIOLOGY IN ASTHMA AND ALLERGY

### *Periostin*

#### **Background**

Periostin (encoded by the gene *POSTN*) is a 92-kDa-secreted N-glycoprotein protein of the fascilin family with homology to FAS1.<sup>4,5</sup> It was originally characterized as a bone development protein,<sup>6</sup> a function reflected in the name periostin,<sup>6</sup> as well as its original name, osteoblast specific factor 2.<sup>4</sup> Periostin is expressed in multiple organs during normal development, tissue remodeling, wound healing, and response to injury (reviewed by Conway and colleagues<sup>7</sup> and Norris and colleagues<sup>8</sup>). It can be induced by several profibrotic stimuli<sup>9</sup> and has been demonstrated to regulate extracellular matrix (ECM) structure and organization through its ability to bind ECM (reviewed by Kii and Ito<sup>10</sup>) and promote collagen fibrillogenesis and cross-linking.<sup>11</sup> As a matricellular signaling protein, periostin can initiate fibrotic and nonfibrotic pathways in a cell-specific and tissue-specific manner.<sup>9</sup>

#### **Periostin expression in normal and diseased tissue**

Aside from its expression in bone and during development, periostin has been observed at sites of injury and regions of epithelial inflammation. In lung, the thickened subepithelial basement membrane of human bronchial asthma samples stained high for periostin<sup>12,13</sup> and subepithelial periostin levels at baseline in asthmatics negatively correlated with subsequent decline of lung function over 20 years.<sup>14</sup> Periostin expression has been observed in other Type 2 helper T-cells or type 2 (T2) immune diseases, including chronic rhinosinusitis with nasal polyps<sup>15,16</sup> and eosinophilic esophagitis.<sup>17</sup> In eosinophilic esophagitis, periostin diminished eosinophil chemotaxis and increased eosinophil adhesion to fibronectin, demonstrating a role for periostin in directly regulating eosinophil accumulation in T2 mucosal tissues.<sup>17</sup> Periostin expression in skin allergic disorders has been examined. Periostin is localized to the papillary dermis in normal and psoriatic skin but is expressed throughout the dermis of lesional skin in atopic dermatitis (AD),<sup>18,19</sup> implying increased IL-13 pathway activation in AD.

The dual function of periostin as an inflammatory and fibrotic pathway protein is reflected in the expression of periostin in areas of active lung fibrosis in idiopathic pulmonary fibrosis (IPF)<sup>20</sup> as well as in the lesional dermis of Systemic Sclerosis subjects, where it correlated with clinical disease activity.<sup>21</sup> In these diseases, fibrosis may be less IL-13 dependent, which may explain the lack of efficacy of anti-IL-13-directed therapy in IPF.<sup>22,23</sup>

#### **Interleukin 4/interleukin 13 pathway activation and periostin induction**

Periostin is down-stream of IL-4 and IL-13 signaling.<sup>24</sup> IL-13-induced or IL-4-induced periostin gene and protein expression is independent of transforming growth factor  $\beta$ .<sup>12</sup> The first studies to demonstrate periostin as a potential biomarker of T2 allergic asthma were 2 publications from Woodruff and colleagues. In a genome-wide microarray on epithelial brushings from asthmatics, smokers, and healthy controls, Woodruff and colleagues<sup>13</sup> showed that *CLCA1*, *POSTN*, and *SERPINE2* were the top genes to distinguish allergic airway disease tissue from healthy. They also confirmed that periostin was released from IL-13-stimulated primary human airway epithelial cells grown in air-liquid interface (ALI). The investigators went on to show that these 3 genes could be used to subset asthmatics into T2 high or T2 low and that T2-high subjects had increased disease severity in terms of elevated airway hyper-responsiveness, IgE, mucus and eosinophils levels, and thickened basement membranes.<sup>25</sup> Silkoff and colleagues<sup>26</sup> examined gene induction from IL-13-treated human airway mucosal cells and identified *CCL26* and *POSTN* as the top differentially

expressed genes, although in this study *CCL26* was superior at segregating T2 asthmatics from non-T2 asthmatics. That periostin was the only gene common to these studies for identifying T2 asthmatics implies its high potential as an IL-13 pathway biomarker.

### ***Circulating periostin levels***

Because airway epithelial cells secrete periostin in a basal direction,<sup>27</sup> it is detectable in the blood, making it a potential biomarker candidate. The development of sensitive ELISAs measuring all splice forms of serum periostin (s-periostin) has allowed circulating levels to be quantified.<sup>28,29</sup> The performance of different periostin assays has been reviewed previously.<sup>30</sup> In serum, periostin is minimally elevated above levels in healthy subjects in general asthmatic populations.<sup>28,31–34</sup> Within T2-high asthma subjects (eg, by high fractional exhaled nitric oxide [FeNO], blood eosinophils, sputum eosinophils, tissue eosinophils, and IgE), however, the results are mixed. Jia and colleagues<sup>29</sup> found s-periostin level was the most sensitive marker to distinguish asthmatic subjects with a high lung eosinophil score from those with a low lung eosinophil score. Conversely, Wagener and colleagues<sup>35</sup> found no correlation between s-periostin and sputum eosinophilia, and s-periostin was not able to distinguish asthma subjects with eosinophilic inflammation from those with noneosinophilic inflammation. These differences may be due to the different assays used or the populations studied because Jia and colleagues used a more severe uncontrolled asthma cohort than Wagener and colleagues.

Elevated s-periostin levels are also associated with other allergic diseases. For example, s-periostin levels were elevated in chronic rhinosinusitis with nasal polyps subjects and could be used to distinguish subjects with and without polyps.<sup>16,36,37</sup> In AD, s-periostin levels correlated with disease severity and decreased after treatment.<sup>18</sup> Psoriatic subjects had lower levels of s-periostin than AD subjects but levels were higher than those in healthy controls.<sup>18,38</sup> In fibrotic indications, similar to what was seen with tissue expression, circulating periostin levels were elevated and s-periostin was predictive of clinical progression in IPF<sup>20</sup> and disease activity in systemic sclerosis,<sup>21</sup> indicating that s-periostin may be a prognostic biomarker for these diseases.

## ***Dipeptidyl Peptidase-4***

### ***Background***

DPP-4 is located on chromosome 2 and encoded by the *DPP4* gene. A 110-kDa glycoprotein, it is a serine protease in the S9 family of enzymes with the capability of cleaving amino-terminal dipeptides that have either L-alanine or L-proline at the penultimate position.<sup>39,40</sup> Alternative names for DPP-4 include DPP-IV, CD26, ADABP, ADCP2, and TP103.<sup>41</sup> DPP-4 can exist as membrane-bound and soluble protein. The soluble form is believed to originate from shedding of the membrane form into plasma.<sup>39</sup>

DPP-4 is more commonly recognized for its enzymatic activity, exemplified by its role in glucose metabolism for inactivating incretins, gastric inhibitory protein (GIP), and glucagon-like peptide-1 (GLP-1).<sup>42</sup> Another important enzymatic function of DPP-4 is in the metabolism of cytokines and chemokines (reviewed by Mortier and colleagues<sup>43</sup>). For example, with regard to asthma and allergy, CCL-11 (eotaxin) is a substrate for DPP-4.<sup>39</sup> This review focuses, however, on the gene and protein expression of DPP-4.

### ***Dipeptidyl peptidase-4 expression in normal and diseased tissue***

DPP-4 was shown to be ubiquitously expressed in kidney, liver, lung, and intestine, at endothelial and epithelial sites. It has also been shown to be expressed on immune

cells including T cells, activated T cells, B cells, natural killer cells, and myeloid cells<sup>39,44</sup> and up-regulated on asthmatic CD4<sup>+</sup> T cells and invariant natural killer T cells.<sup>44</sup> As such, DPP-4 has been described as a surface antigen in T-cell activation and regulation, with potential involvement in various immune and neuroendocrine functions.<sup>39,45</sup>

In airway studies, the *DPP-4* gene was differentially expressed in nasal and bronchial transcriptomes from atopic asthmatics relative to nonatopic controls.<sup>46</sup> Immunostaining for DPP-4 protein was enhanced in airway bronchial epithelial cells (BECs) from steroid naïve relative to steroid-treated asthmatics.<sup>47</sup> These data are compatible with the observed effect of corticosteroids on serum DPP-4 (discussed later).

DPP-4 staining was increased in inflamed skin lesions from AD subjects compared with healthy nonatopic controls.<sup>48</sup> Moderate DPP-4 staining was seen in normal skin and localized to the dermis compared with AD skin, where both the dermis and epidermis were DPP-4 positive with particularly strong staining in the epidermis. The dermal DPP-4 staining in the AD skin, although lower than in the epidermis, was still elevated above the levels seen in healthy dermis. In contact dermatitis skin, DPP-4 staining was observed in the epidermis in a similar pattern to that observed in AD.<sup>48</sup>

#### ***Interleukin 4/interleukin 13 pathway activation and dipeptidyl peptidase-4 induction***

DPP-4 has been shown to be induced by IL-13 in multiple models of asthma and allergy.<sup>47,49–52</sup> Similar to periostin, profibrotic molecules can also induce DPP-4 expression.<sup>53</sup> Furthermore, IL-13 gene expression positively correlated with *DPP4* mRNA expression in asthma patients on ICS and oral corticosteroids (OCS).<sup>49,50</sup> *DPP4* was also shown to be in the top 12 genes that positively correlated with *IL13* mRNA expression in transcriptomic studies of nasal epithelial cells and BECs from children with asthma.<sup>46</sup>

#### ***Circulating dipeptidyl peptidase-4 levels***

Although early studies reported elevated serum DPP-4 levels in asthmatics relative to healthy subjects and no effect of ICS treatment,<sup>44</sup> more recent studies, with larger subject numbers, have contradicted both these observations. Serum DPP-4 protein concentrations were increased in patients with allergic asthma compared with healthy age-matched controls.<sup>39</sup> Furthermore, another study reported that healthy individuals had higher serum DPP-4 levels than severe asthmatics on ICS therapy.<sup>54</sup> Moreover, serum DPP-4 protein levels were lower in corticosteroid treated compared with corticosteroid-naïve patients with asthma.<sup>47</sup> Similarly, early correlations between serum DPP-4 levels and blood eosinophils have not reproduced in larger patient data sets, where no association of serum DPP-4 with IgE, forced expiratory volume in the first second of expiration (FEV<sub>1</sub>), FeNO, and blood or sputum eosinophils was found.<sup>55</sup> Serum DPP-4 levels were inversely correlated with blood neutrophils in patients with severe airway disease.<sup>55</sup>

#### ***Summary***

Periostin and DPP-4 show similarities in that they can both be induced by IL-13 and IL-4<sup>24</sup> and by other profibrotic stimuli.<sup>9,53</sup> This, however, may reduce their specificity as biomarkers of IL-13 pathway activity, which needs to be considered when using them for guiding treatment with anti-IL-13-directed therapies (because they may be elevated in patients with evidence of other concomitant fibrotic disease). Both periostin and DPP-4 gene and protein expression, however, are increased in patients with T2-associated diseases, including allergic asthma and AD, and both markers

decrease with corticosteroid therapy. These data also imply that they are downstream of IL-13 pathway signaling in T2-associated diseases and can be markers of T2 disease and may, therefore, be candidate biomarkers of anti-IL-13-directed therapy.

## DEMOGRAPHY/DISEASE

The ideal biomarker, as well as a measurable molecule that relates to disease status, should also be unaffected by other biological variables that could be present in the target patient population. Demographics, such as age and gender, or disease comorbidities may affect a biomarker and as such it is important to understand these relationships before a biomarker is used in the clinic.

### *Effects of Age and Gender on Periostin*

Given its connection to bone metabolism, multiple studies have looked for gender or age differences in s-periostin levels. Overall, s-periostin was not different between men and women,<sup>16,28,31,33,56</sup> except in postmenopausal women with additional osteoporosis risk factors.<sup>57</sup> It has also been reported that s-periostin levels are equivalent in adult subjects across a range of ages<sup>28,33,56,58</sup> although 1 group reported higher s-periostin in subjects aged over 60, a presumed feedback loop in response to age-related bone catabolism.<sup>31</sup> Notably, s-periostin is elevated in teenage subjects relative to adults.<sup>28,58</sup> Furthermore, elevated levels of s-periostin in children ages 6 years to 11 years masked any increase due to allergic disease status.<sup>58</sup> Although unproved, the hypothesis is that active bone growth is the source of the raised s-periostin concentration in children and adolescents.<sup>21,33</sup> This may complicate the general use of periostin as a predictive biomarker for anti-IL-13-directed therapy, requiring different thresholds for defining s-periostin-high patients for therapy in adolescents compared with s-periostin-high patients for therapy in adults.

### *Effects of Body Mass Index Status on Periostin*

Further potential confounders for use in predicting response to IL-13 pathway-directed therapy include BMI,<sup>31,34</sup> which was found to be inversely correlated with s-periostin levels independently of asthma status. Obesity is associated with cardiovascular and metabolic disease. Despite periostin's involvement in cardiac development, however, the association with cardiac complications has not been fully explored; a small study found no association with hypertension or heart disease.<sup>33</sup> Therefore, s-periostin may not have the equivalent strong association with metabolic disease and obesity that DPP-4 has (discussed later).

### *Effects of Cancer on Periostin*

Both periostin and DPP-4 have been linked to cancer malignancies. Because both are pleiotropic proteins, this relationship could be the result of multiple mechanisms; epithelial mesenchymal transition, angiogenesis, and integrin activation in the case of periostin<sup>59,60</sup> or cell proliferation, migration, and adhesion for DPP-4.<sup>61,62</sup> The shared capacity to bind and organize ECM would also provide a direct effect, whereby these proteins may modify the tumor microenvironment and control metastasis. Despite this potential, these proteins may still have utility as biomarkers of IL-13 biology in T2 diseases. The prevalence of cancer is expected to be low in this population,<sup>63,64</sup> and, because cancer is usually an exclusion criterion in controlled clinical trials, this concern should not confound the interpretation of studies using periostin or DPP-4 as biomarkers to predict response.

### ***Periostin Associations with Other Type 2 Biomarkers***

A complete understanding of how a biomarker relates to other disease metrics is also important. In terms of a direct measure of airway inflammation, FeNO is an alternative noninvasive biomarker of T2 airway inflammation. FeNO is also downstream of IL-13 activation (reviewed by Ricciardolo and Silkoff<sup>65</sup>). Because both FeNO and periostin should reflect IL-13 pathway activity, they would be expected to correlate. Silkoff and colleagues<sup>26</sup> showed that 80% of their periostin-high asthma subjects were also FeNO high. The lack of complete correlation likely reflects the incomplete selectivity of periostin (and FeNO for that matter) for IL-13. Other surrogate markers have been used to monitor T2 inflammation, including sputum or blood eosinophils. Weak, but significant, positive associations have been observed between serum and peripheral blood eosinophils.<sup>29,31,33,66</sup> This may be due, however, to the requirement for further segmentation of asthma patients with eosinophilic inflammation.<sup>29,35</sup> For example, although IL-13 can induce eosinophilic inflammation, there is a subpopulation of severe asthmatic patients with late-onset disease where IL-5 is the dominant cytokine for their eosinophilia<sup>67</sup> and these patients have low atopy. IgE, but not atopy, correlated with s-periostin levels.<sup>31,33</sup> Higher s-periostin concentrations were associated with elevated asthma exacerbation rate, but there have been mixed associations for s-periostin with changes in lung function (FEV<sub>1</sub> and forced vital capacity).<sup>29,31,33,68</sup>

Although the correlations are not perfect, these observations indicate the use of s-periostin as an alternative to FeNO, high IgE, and high blood eosinophils as a potential biomarker of T2 disease.

### ***Effects of Corticosteroids on Periostin***

Because many severe asthma subjects are on corticosteroids, the effect of this treatment on a biomarker must be assessed. Periostin gene expression was examined in airway epithelial cells from asthmatics prior to or after treatment with inhaled fluticasone.<sup>13</sup> Periostin gene expression was elevated in asthmatics (relative to healthy controls) and after fluticasone treatment *POSTN* expression was down-regulated relative to asthmatic subjects on placebo.<sup>13</sup> This finding was confirmed in vitro, where IL-13 stimulated *POSTN* expression in primary human airway epithelial cells cultured in ALI was inhibited by addition of dexamethasone or budesonide. Furthermore, high baseline expression of *POSTN* in airway epithelial brushings predicted improvements in FEV<sub>1</sub> at 4 weeks in the asthmatic subjects randomized to inhaled fluticasone.<sup>13</sup> When these data were reanalyzed using a 3-gene signature (*POSTN*, *CLCA1*, and *SERPINB2*) to define T2-high versus T2-low subjects, the T2-high cohorts showed significant improvement in FEV<sub>1</sub> at 4 weeks and 8 weeks of fluticasone treatment compared with T2-low subjects on fluticasone or placebo-treated asthmatics.<sup>25</sup> These findings demonstrate the sensitivity of periostin to corticosteroids but also its potential for guiding treatment of T2-high patients.

### ***Effects of Age and Gender on Dipeptidyl Peptidase-4***

There were 2 healthy population studies that reported that serum DPP-4 protein concentrations were higher in adolescents compared with adults.<sup>54,69</sup> Individuals ages 16 years to 21 years had the highest circulating DPP-4 levels, which subsequently decreased with advancing age.<sup>54</sup> With regard to differences in circulating DPP-4 protein among genders in the healthy population, 1 investigation reported that serum DPP-4 levels were higher in men compared with women.<sup>54</sup> In another study plasma and serum DPP-4 levels in healthy men trended slightly higher than healthy women

but were not statistically different between genders in another study.<sup>69</sup> When matched for BMI status, male and female plasma DPP-4 levels were equivalent.<sup>70</sup>

### ***Effects of Body Mass Index Status on Dipeptidyl Peptidase-4***

There are conflicting data on the relationship of DPP-4 with BMI status. One study reported that serum DPP-4 levels did not change with BMI status,<sup>54</sup> whereas a second study showed plasma DPP-4 protein levels correlated with BMI higher in obese patients than normal weight individuals.<sup>70</sup>

### ***Dipeptidyl Peptidase-4 Associations with Other Type 2 Biomarkers***

There are few data available evaluating the relationship of serum DPP-4 with other T2 biomarkers. In 1 study, serum DPP-4 levels in asthmatics correlated with blood eosinophils<sup>44</sup> whereas in a more recent, larger study serum DPP-4 protein concentrations in asthma did not correlate with blood eosinophils or s-periostin.<sup>50</sup> *DPP4* mRNA expression from BECs and bronchial brushings from asthma patients on ICS and OCS, however, correlated positively with FeNO.<sup>47,49,50</sup> Because DPP-4 did not correlate with s-periostin, this suggests that DPP-4 may inform on different aspects of IL-13 pathway activation and, therefore, may provide an alternative biomarker for identifying patients to direct anti-IL-13 therapy.

### ***Effects of Corticosteroids on Dipeptidyl Peptidase-4***

In experimental studies, mometasone was shown to suppress IL-13 induced DPP-4 expression in BECs cultured in ALI.<sup>51</sup> In another study, DPP-4 protein was shown to be reduced in serum and BECs, along with mRNA expression in bronchial epithelium, from patients with asthma on ICS compared with steroid-naïve asthmatics.<sup>47</sup> Other studies have also demonstrated that serum DPP-4 protein levels were reduced in asthma patients with severe disease who were on corticosteroid regimens compared with healthy controls.<sup>54</sup>

These observations indicate that DPP-4, like IL-13, can be modulated by corticosteroid treatment. It is unknown, however, whether this is a direct effect of corticosteroids on DPP-4 expression or a downstream effect due to reduction in IL-13 expression. Although it has been shown that both periostin and DPP-4 can be modulated by corticosteroids, the use of biologic anti-IL-13-directed therapies would be used in severe uncontrolled asthma patients who demonstrate evidence of raised serum concentrations of these molecules despite receiving high-dosage corticosteroid therapy.

### ***Other Drugs That May Have an Impact on Circulating Dipeptidyl Peptidase-4 Concentrations***

As discussed previously, DPP-4 is involved in glucose metabolism by inactivating incretins, GIP and GLP-1, and is a target for treatment of patients with T2 diabetes by inhibiting its enzymatic activity.<sup>42</sup> The impact of DPP-4 inhibitors has been studied for effects on DPP-4 protein levels in serum. One study showed that there was an increase in plasma DPP-4 levels in diabetic patients treated with sitagliptin compared with predose levels.<sup>71</sup> Hence, the use of DPP-4 inhibitors may also confound the potential to determine protein DPP-4 concentrations for anti-IL-13-directed therapy.

### ***Summary***

When comparing these biomarkers by demographic characteristics and other T2 markers, differences begin to be seen between them. Both markers show similarities in relationship of serum levels with age, higher in adolescents than adults. This is where the similarities, however, seem to end. Periostin was shown to inversely

correlate with BMI, whereas DPP-4 demonstrated either no association or a positive correlation with BMI. This may be due to DPP-4 also being a marker of metabolic disease, therefore reflecting glucose metabolism activity.

Of particular interest is the difference in the relationship of these biomarkers with other T2 biomarkers. Serum levels of periostin have shown positive relationships with FeNO, blood eosinophils, and serum IgE. Serum levels of DPP-4 have shown no correlation with blood eosinophils or s-periostin. Local expression of DPP-4 in the airway, however, did show a relationship with FeNO in asthmatics. It is possible that serum DPP-4 may represent other components of IL-13 biology or that serum levels of DPP-4 are indicative of both T2 disease activity and glucose metabolism activity, whereas local airway expression may be more reflective of local T2 pathway activation. These differences may have an impact on periostin's and DPP-4's capacity to predict response to anti-IL-13-directed therapy.

## **SERUM PERIOSTIN AND DIPEPTIDYL PEPTIDASE-4 TO DIRECT TREATMENT IN ASTHMA AND ALLERGIC DISEASE**

### ***Studies with Serum Periostin***

Because asthma is a heterogeneous disease, a biomarker may be required to identify those patients with evidence of IL-13 pathway activation to direct anti-IL-13-therapy. Lebrikizumab, tralokinumab, and dupilumab are 3 monoclonal antibodies that can inhibit the IL-13 pathway in asthma and allergic disease.

Lebrikizumab is a humanized IgG<sub>4</sub> monoclonal antibody that specifically binds to IL-13 and inhibits its function.<sup>72</sup> In an initial phase 2 randomized, placebo-controlled, clinical study conducted in moderate to severe uncontrolled asthmatic patients taking inhaled corticosteroids, lebrikizumab was dosed monthly for 6 months. In this study, the primary endpoint was change in prebronchodilator FEV<sub>1</sub> from baseline to week 12. In the total study population, treatment with lebrikizumab demonstrated a statistically significant improvement in FEV<sub>1</sub> compared with placebo at week 12. When patients were subdivided into those with high or low s-periostin at baseline (the cutoff value was the study population median concentration of periostin), however, there was a greater effect on improvement in FEV<sub>1</sub> at week 12 compared with placebo in those with high compared with those with low s-periostin levels.<sup>73</sup> This initial clinical study confirmed the hypothesis that patients with evidence of IL-13 pathway activation would have greater benefit from anti-IL-13 therapy and further that s-periostin might be useful as a biomarker to identify such patients. Two further dose-ranging studies in patients with uncontrolled asthma despite using medium-dose to high-dose ICS plus a second controller medication demonstrated greater efficacy in s-periostin-high compared with s-periostin-low patients, this time on a primary endpoint of rate of asthma exacerbations during a median treatment duration of 24 weeks. This was a striking difference of a 60% reduction in s-periostin-high patients compared with a 5% reduction in s-periostin-low patients.<sup>68</sup> In a dose-ranging study in mild asthmatics not taking inhaled corticosteroids, where the primary endpoint was change from baseline in FEV<sub>1</sub>, there was a numerically greater but clinically and statistically insignificant change in FEV<sub>1</sub> compared with placebo and there was no difference when subgrouping patients by baseline s-periostin levels.<sup>22</sup> This outcome may be because the patient population in this study was too mild to detect changes in lung function.

The lebrikizumab asthma program culminated in 2 phase 3 studies conducted in adult, uncontrolled severe asthmatics on high-dosage ICS plus at least 1 additional controller medication, LAVOLTA I and LAVOLTA II. These studies consisted of a 52-week treatment period with 2 dose regimens of lebrikizumab compared with

placebo and with patients stratified to treatment based on baseline s-periostin concentration. The primary endpoint was rate of exacerbations; however, the primary population for analysis was biomarker-high patients, defined as periostin greater than or equal to 50 ng/mL or blood eosinophils greater than or equal to 300 cells/ $\mu$ L. Although LAVOLTA I demonstrated a statistically and clinically meaningful improvement on annual asthma exacerbation rate (AAER) compared with placebo with both lebrikizumab doses, LAVOLTA II missed statistical significance. In the secondary analyses, both studies demonstrated statistically and clinically significant improvements in asthma exacerbation rate compared with placebo in patients who were s-periostin high but inconsistent efficacy in patients who were s-periostin low.<sup>74</sup> Extending the primary analysis population to include patients with high blood eosinophil or high s-periostin may have included a more heterogeneous group of patients, because although IL-13 can induce eosinophilia, there are patients with high blood eosinophils with late-onset disease who are more dependent on IL-5 and less atopic.<sup>67</sup> The utility of s-periostin to predict efficacy with anti-IL-13 therapy was confirmed in these trials.

Tralokinumab is a human IgG<sub>4</sub> monoclonal antibody that neutralizes IL-13.<sup>75</sup> In an initial phase 2a study in patients with moderate to severe asthma and a primary endpoint of change from baseline in mean Asthma Control Questionnaire (ACQ)-6 score, a 12-week treatment period with tralokinumab showed no statistically significant difference compared with placebo. In a subgroup of patients, however, where sputum IL-13 levels were measured, patients with high sputum IL-13 showed greater changes in ACQ-6 and FEV<sub>1</sub> compared with those with low sputum IL-13. A later phase 2b, randomized, placebo-controlled study investigating 2 dosing regimens of tralokinumab in adult patients with severe uncontrolled asthma with treatment for 1 year and a primary endpoint of AAER evaluated the concept of s-periostin to identify patients who would gain benefit from treatment with tralokinumab.<sup>76</sup> There was no difference between tralokinumab treatment and placebo on exacerbation rate reduction or secondary endpoints of change in ACQ-6 or Asthma Quality of Life Questionnaire (AQLQ) in the intention-to-treat (ITT) population.<sup>76</sup> At week 52, however, there was a significant increase in FEV<sub>1</sub> compared with placebo in patients receiving tralokinumab every 2 weeks.

Subgrouping patients by s-periostin high and s-periostin low (using study population median concentration as a cutpoint), showed a numerically greater but statistically nonsignificant reduction in exacerbation rate in patients treated with tralokinumab compared with the effect in the ITT study population in patients with high s-periostin at baseline but not in patients with low s-periostin. Increases in FEV<sub>1</sub> at week 52 were similar between patients in the high-periostin and low-periostin subgroups receiving tralokinumab versus placebo. Improvement in ACQ-6 was greater in s-periostin-high patients compared with s-periostin-low patients but not statistically significant, and there was no difference in AQLQ improvement.

In a post hoc analysis with further subsetting, it was shown in a subgroup of patients not receiving chronic OCS therapy and with airway reversibility at baseline that there were further numerical and statistically significant improvements in exacerbation rate, FEV<sub>1</sub>, and ACQ-6 but not AQLQ in patients with high s-periostin but not low s-periostin. These studies with tralokinumab again highlighted the need for directed therapy for asthma patients based on a biomarker to identify those patients with evidence of IL-13 pathway activation.

The phase 3 program with tralokinumab included 2 pivotal studies in patients with severe uncontrolled asthma, despite treatment with ICS plus long-acting  $\beta_2$ -agonists, with demonstrated postbronchodilator reversibility to short-acting  $\beta_2$ -agonists and not on chronic OCS therapy. STRATOS 1 (NCT02161757) and STRATOS 2 (NCT02194699)

were both designed with the primary outcome of annual AAER.<sup>77</sup> Patients were stratified at randomization in both studies by baseline s-periostin levels (<16.44 ng/mL or greater than or equal to 16.44 ng/mL; corresponding to the median value observed in previous studies). The studies were run concurrently but analyzed sequentially. The aim was to use the results from STRATOS 1 to identify a target population with enhanced response to treatment with tralokinumab based on baseline levels of biomarkers associated with IL-13 pathway activation, including s-periostin, DPP-4, FeNO, and total serum IgE. The results from STRATOS 1 determined the biomarker-positive population to be assessed in STRATOS 2.<sup>77</sup> AstraZeneca (Cambridge, U.K.) has announced that STRATOS 1 and STRATOS 2 both failed to achieve their respective primary endpoints.<sup>78,79</sup> In STRATOS 1, the primary endpoint of a significant reduction in AAER in the overall study population was not met, but it was reported that a clinically relevant reduction in AAER was observed in a subpopulation of patients with an elevated biomarker associated with increased IL-13 activity, which would be the focus for the analysis of STRATOS 2.<sup>78</sup> The later announcement of STRATOS 2 reported that the biomarker selected for the subpopulation analysis was patients with elevated FeNO, but that the primary endpoint was not met.<sup>79</sup> At the time of writing this review, no data have been reported on the performance of s-periostin and serum DPP-4 in these studies.

Dupilumab is a fully human monoclonal antibody directed against the IL-4 receptor alpha subunit and inhibits both IL-4 and IL-13 signaling.<sup>80</sup> In a phase 2b study in uncontrolled, moderate to severe asthmatics, where the primary analysis population was patients with blood eosinophils greater than or equal to 300 cells/ $\mu$ L, dupilumab showed statistically significant improvements in lung function, exacerbation rate, ACQ-5, and AQLQ. Similar changes were also observed, however, in the total study population and patients with blood eosinophils less than 300 cells/ $\mu$ L, suggesting efficacy in a wide range of patients. A post hoc analysis also showed that there was efficacy in patients who were s-periostin high and s-periostin low, although efficacy was numerically greater in those patients who were s-periostin high.<sup>81</sup> These findings were in contrast to the data with the monoclonal antibodies that target IL-13, suggesting that combined targeting of IL-4 and IL-13 may have a broader therapeutic role in severe asthma.

Dupilumab has also demonstrated efficacy in AD and nasal polyposis, but there has been no evaluation of efficacy by biomarkers in these diseases.<sup>82,83</sup>

Finally, the effect of s-periostin to predict efficacy in treating patients with omalizumab has also been evaluated. Omalizumab is a humanized IgG1 $\kappa$  monoclonal antibody that specifically binds to free human IgE.<sup>84</sup> In patients with uncontrolled severe persistent allergic asthma, analyses were performed evaluating treatment effects to T2 biomarkers that included baseline s-periostin. After 48 weeks of omalizumab, reductions in protocol-defined exacerbations were greater in high s-periostin versus low s-periostin.<sup>85</sup> Therefore, periostin may also be a biomarker for other T2-directed therapies. This is perhaps not surprising, because IL-13 can direct class switching of immunoglobulin production to IgE from B cells.<sup>1</sup>

## ***Studies with Serum Dipeptidyl Peptidase-4***

### ***Asthma***

Currently, available data on the potential utility of DPP-4 as a predictive biomarker for anti-IL-13 treatment in asthma is limited to the same phase 2b study with tralokinumab that also investigated the utility of s-periostin (described previously<sup>76</sup>). As discussed previously, although serum DPP-4 concentrations were lower in patients with asthma than healthy volunteers, some patients had high concentrations despite corticosteroid therapy. Therefore, a post hoc analysis was conducted that

hypothesized elevated serum DPP-4, despite high corticosteroid dosages, may identify patients who benefit from tralokinumab.

Similar to results observed subsetting by s-periostin levels, there were greater reductions in annual exacerbation rate in DPP-4 high than DPP-4 low; however, these reductions were not significant compared with placebo. Unlike that observed by s-periostin subgrouping, numerically greater and significant improvements in prebronchodilator FEV<sub>1</sub> were seen in the DPP-4-high subset but not in DPP-4-low subset. Furthermore, in contrast to the high s-periostin group, numerically greater and statistically significant improvements in ACQ-6 and AQLQ compared with placebo at week 52 were seen in patients receiving tralokinumab in the DPP-4-high subgroup. In the DPP-4-low subset, all changes were of a lower magnitude than the DPP-4-high subgroup and none was statistically significant.

Further subsetting of patients investigated serum DPP-4-high and DPP-4-low patients receiving tralokinumab with airway reversibility at baseline but not receiving long-term OCS. This post hoc analysis revealed further improvements in the magnitude of reduction of AAER rate versus placebo, but again these did not achieve statistical significance, probably due to the low subject number.

Within this subset there were also further improvements in FEV<sub>1</sub>, ACQ-6, and AQLQ in those who were DPP-4 high, which achieved statistical significance. In the DPP-4-low group, again changes were small and no outcomes achieved statistical significance. The data in those who were DPP-4 high are again in contrast to those who were s-periostin high, which showed no significant improvement in AQLQ.

### ***Atopic dermatitis***

Currently, available data on the potential utility of DPP-4 as a predictive biomarker for anti-IL-13 treatment in adults with moderate to severe AD is limited to a single phase 2b study with tralokinumab.<sup>86</sup> The coprimary endpoints were change from baseline in total Eczema Area and Severity Index (EASI) and percentage of Investigator's Global Assessment (IGA) responders (patients achieving an IGA of 0 or 1 and at least a 2-grade reduction from baseline) at week 12. Tralokinumab at 150 mg and 300 mg in the ITT population significantly reduced total EASI from baseline and a greater number of patients had an IGA response of 0 or 1 compared with placebo.

In an exploratory analysis, patients were subgrouped by median baseline serum DPP-4 to DPP-4 high and DPP-4 low. In summary, DPP-4-high moderate to severe AD patients demonstrated significant efficacy in both primary endpoints compared with placebo with effect sizes greater than in the ITT population at week 12 after treatment with 300 mg tralokinumab. No significant differences in either primary endpoint was observed for the DPP-4-low subgroup. These data suggest serum DPP-4 may also serve as a predictive biomarker for AD patients who may benefit from tralokinumab treatment.

### ***Summary***

Subgrouping severe asthma patients by baseline levels of s-periostin or serum DPP-4 into biomarker high and low subpopulations demonstrated greater efficacy on clinical efficacy endpoints in the biomarker high subgroup compared with biomarker low for treatment with anti-IL-13 monoclonal antibodies. For s-periostin, this was despite different assay formats that were used for trials with lebrikizumab<sup>74</sup> and tralokinumab.<sup>76</sup>

In the phase 2b clinical study with tralokinumab that explored the use of both s-periostin and serum DPP-4, there were differences in how each biomarker performed to predict efficacy response. In this study, s-periostin-high patients showed numerically greater magnitude of effect on exacerbation rate reduction but lower magnitudes of

effect on improvements in FEV<sub>1</sub> and ACQ-6 and little to no effect on AQLQ compared with the efficacy responses in the subgroup of patients who were serum DPP-4 high. This may indicate that s-periostin and DPP-4 have different biology associated with IL-13 activation and therefore can inform on different efficacy endpoints. The results using s-periostin to predict efficacy with omalizumab (anti-IgE) therapy also indicate that s-periostin may be a biomarker for predicting response to other T2-directed therapies. Furthermore, the data with serum DPP-4 in AD also suggest these biomarkers may have utility in other T2 diseases as well as asthma.

## OVERALL SUMMARY

Periostin and DPP-4 are both induced by the T2 cytokines IL-4 and IL-13 and have increased local expression in T2 disease tissue, including lung, nasal, and skin. Furthermore, they are measurable in serum, supporting their potential for practical application in the clinical setting. Both markers share similar factors that can potentially confound their specificity as markers of T2 inflammation with both induced by profibrotic factors and also being increased in the serum of adolescents compared with adults.

There are also striking differences between these 2 serum biomarkers, however, particularly with respect to their relationship with other T2 biomarkers. In general, periostin shows correlations with other T2 markers, unlike DPP-4 that shows no relationship. Furthermore, there is no correlation between s-periostin and serum DPP-4.

In clinical trials of severe, uncontrolled asthma, periostin has consistently demonstrated enhanced efficacy of anti-IL-13 therapy in patients with high serum levels. The available data with serum DPP-4 are more limited, restricted to 1 study with tralokinumab in asthma and 1 in AD. Patients with high DPP-4, however, also showed enhanced efficacy to treatment with tralokinumab in both asthma and AD.

High s-periostin levels also showed enhanced efficacy to treatment with omalizumab, indicating that periostin may also have application as a more general marker of T2 inflammatory disease.

The failure of lebrikizumab and tralokinumab to meet the primary endpoints in their respective phase 3 asthma programs may suggest there is little need for biomarkers for anti-IL-13-directed therapy in asthma, especially because dupilumab has shown efficacy in s-periostin-high and s-periostin-low subgroups. Despite this, they may still be of value in understanding the pathologic mechanisms of severe asthma.

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