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# Development and analytical performance of a new ARCHITECT automated dipeptidyl peptidase-4 immunoassay

Philip M. Hemken[a,1], Nicolette M. Jeanblanc[a,1], Tracey Rae[a], Susan E. Brophy[a], Maria J. Datwyler[a], Ying Xu[a], T. Scott Manetz[b], Inna Vainshtein[c], Meina Liang[c], Xiaodong Xiao[d,2], Partha S. Chowdhury[d,3], Chien-ying Chang[d], Katie Streicher[e], Lydia Greenlees[e], Koustubh Ranade[e], Gerard J. Davis[a,*]

[a] Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA
[b] Translational Sciences, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA
[c] Clinical Pharmacology and DMPK, MedImmune, 319 N Bernardo Ave, Mountain View, CA 94043, USA
[d] Antibody Discovery and Protein Engineering, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA
[e] Translational Medicine, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA

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## ABSTRACT

*Background:* Dipeptidyl peptidase-4 (DPP-4) may be a suitable biomarker to identify people with severe asthma who have greater activation of the interleukin-13 (IL-13) pathway and may therefore benefit from IL-13-targeted treatments. We report the analytical performance of an Investigational Use Only immunoassay and provide data on the biological range of DPP-4 concentrations.
*Methods:* We assessed assay performance, utilising analyses of precision, linearity and sensitivity; interference from common endogenous assay interferents, and from asthma and anti-diabetic medications, were also assessed. The assay was used to measure the range of serum DPP-4 concentrations in healthy volunteers and subjects with diabetes and severe, uncontrolled asthma.
*Results:* The total precision of DPP-4 concentration measurement (determined using percentage coefficient of variation) was ≤5% over 20 days. Dilution analysis yielded linear results from 30 to 1305 ng/mL; the limit of quantitation was 19.2 ng/mL. No notable endogenous or drug interferences were observed at the expected therapeutic concentration. Median DPP-4 concentrations in healthy volunteers and subjects with asthma or Type 1 diabetes were assessed, with concentrations remaining similar in subjects with diabetes and asthma across different demographics.
*Conclusion:* These analyses indicate that the ARCHITECT DPP-4 Immunoassay is a reliable and robust method for measuring serum DPP-4 concentration.

*Abbreviations:* BGG, bovine gamma globulin; BMI, body mass index; CI, confidence interval; CLSI, Clinical Laboratory Standards Institute; CV, coefficient of variation; DPP-4, dipeptidyl peptidase-4; HAMA, human anti-mouse antibodies; Ig, immunoglobulin; IL-13, interleukin-13; IUO, Investigational Use Only; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; mAb, monoclonal antibody; PI, prediction interval; RF, rheumatoid factor; RLU, relative light units; SRT, serum tube-red top; SST, serum separator tube; $Th_2$, T-helper-2

* Correspondence to: Dept 09CD, Bdg AP20/1, 100 Abbott Park Road, Abbott Park, IL 60064, USA.
*E-mail addresses:* philip.hemken@abbott.com (P.M. Hemken), nicolette.jeanblanc@abbott.com (N.M. Jeanblanc), Tracey.Rae@abbott.com (T. Rae), susan.brophy@abbott.com (S.E. Brophy), maria.datwyler@abbott.com (M.J. Datwyler), ying.xu1@abbott.com (Y. Xu), ManetzS@medimmune.com (T.S. Manetz), vainshteini@medimmune.com (I. Vainshtein), LiangM@medimmune.com (M. Liang), xiaodong.xiao@bms.com (X. Xiao), Partha.Chowdhury@sanofi.com (P.S. Chowdhury), changc@medimmune.com (C.-y. Chang), StreicherK@medimmune.com (K. Streicher), GreenleesL@medimmune.com (L. Greenlees), RanadeK@medimmune.com (K. Ranade), gerard.davis@abbott.com (G.J. Davis).
[1] Joint first authors.
[2] Present address: Antibody Discovery, Bristol-Myers Squibb, 700 Bay Road, Redwood City, CA 94063, USA.
[3] Present address: Biologics Discovery, Sanofi, 49 New York Avenue, Framingham, MA 01701, USA.

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## 1. Introduction

Interleukin-13 (IL-13) is a cytokine, secreted in large quantities by $CD4^+$ T-helper-2 ($Th_2$) cells in patients with $Th_2$-driven asthma or eosinophilic inflammation [1]. Increased IL-13 mRNA expression and protein concentration in bronchial biopsies, sputum and bronchoalveolar lavage fluid from patients with asthma, compared with healthy individuals, supports a role for IL-13 in the pathophysiology of some types of asthma [2–5]. Furthermore, sputum IL-13 concentrations and the number of cells expressing IL-13 in the bronchial submucosa and airway smooth muscle bundle have been shown to be increased in people with severe asthma [6]. Consequently, identifying people with uncontrolled asthma and increased IL-13 activity might aid in selection of patients who may benefit from anti-IL-13–targeted therapy [7–10].

During $Th_2$–driven inflammation, IL-13 is expressed locally in inflamed tissue and is present in serum in low concentrations [7,11]. This creates a challenge for its use as a biomarker of asthma, particularly as serum IL-13 concentrations remain similar between healthy volunteers and people with asthma [11]. An alternative, readily measureable biomarker is therefore required in order to identify those people with IL-13–driven asthma. Dipeptidyl peptidase-4 (DPP-4) production by airway cells is induced, in vitro, by IL-13 [8]. *DPP4* gene expression has been shown to be upregulated in the nose and bronchi of children with asthma and in the bronchi of adults with asthma, which also correlated with IL-13 mRNA upregulation [12,13]. Therefore, DPP-4 may prove to be a suitable biomarker for identifying people with IL-13-driven asthma who could benefit from IL-13-targeted treatments. Indeed, the relationship between serum DPP-4 concentrations and response to an anti–IL-13–targeted treatment has previously been shown in a Phase IIb study of tralokinumab, an anti–IL-13 monoclonal antibody (mAb) in subjects with severe, uncontrolled asthma [14].

DPP-4 (also known as adenosine deaminase complexing protein 2 or CD26) is a 766-amino acid membrane serine peptidase, highly expressed in the lung, kidney, liver and small intestines [15]. It is an integral type II glycoprotein homodimer anchored to the cell membrane by its signal peptide [15]. DPP-4 can be shed from the cell membrane into circulation in a soluble, active form [15], facilitating its measurement as a soluble biomarker. DPP-4 regulates glucose metabolism through degradation of incretin peptides [16,17] and may also have enzymatic functions in immune system modulation, cardiovascular physiology and tumour biology [18–21].

We describe the development of the ARCHITECT DPP-4 Investigational Use Only (IUO) Immunoassay, currently in use to assess the utility of DPP-4 as a biomarker in Phase III studies investigating tralokinumab in subjects with severe uncontrolled asthma (NCT02161757, NCT02194699 [10]). We report the analytical performance of the assay and provide data on the biological variability of serum DPP-4 concentrations across different subject demographics.

## 2. Materials and methods

### 2.1. Assay description

The IUO ARCHITECT DPP-4 Immunoassay was developed for use with the ARCHITECT Immunoassay *i* System (Abbott Laboratories, Abbott Park, IL) [22]. The assay determines serum DPP-4 concentration using a two-step dual non-competing mAb sandwich process with methodology that has previously been described [23]. Briefly, assay samples and standards were diluted 10-fold with line diluent and microparticles; DPP-4 was captured by rat anti–DPP-4 mAb-coated paramagnetic microparticles and detected with acridinium-labelled mouse anti–DPP-4 mAb. A chemiluminescent signal, reportable as relative light units, directly correlates with the amount of DPP-4 present (Fig. 1). The mAbs used in the immunoassay were generated by MedImmune (Gaithersburg, MD) using a hybridoma platform and purified using affinity chromatography with Protein G and Protein A for the rat and mouse mAb, respectively [24].

The assay was standardised using a commercially available purified recombinant human dimeric DPP-4 protein (NCBI accession number: CAA43118), with a C-terminal His-tag for purification (Bio-Techne Inc., MN, USA), produced from a mouse myeloma-derived NS0 cell line. The assay had a calibration range of 0–1000 ng/mL, selected to reflect the baseline concentrations of DPP-4, prior to treatment with agents, of the population for which the assay is intended. The assay utilised subjects in a Phase IIb study of

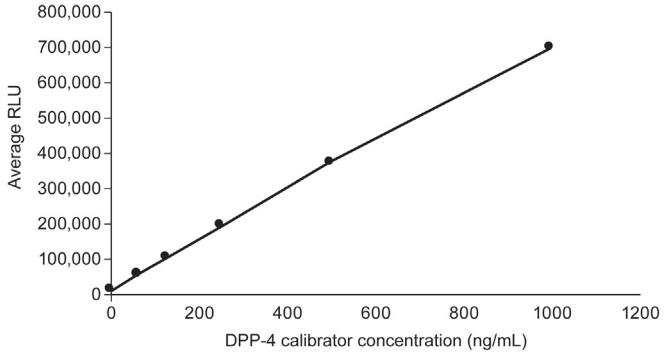


**Fig. 1.** Relationship between relative light units (RLU) and dipeptidyl peptidase-4 (DPP-4) concentrations.