APPLICATION NOTE

Noninvasive, *in vivo* quantitation of asthma severity using fluorescence molecular tomography.

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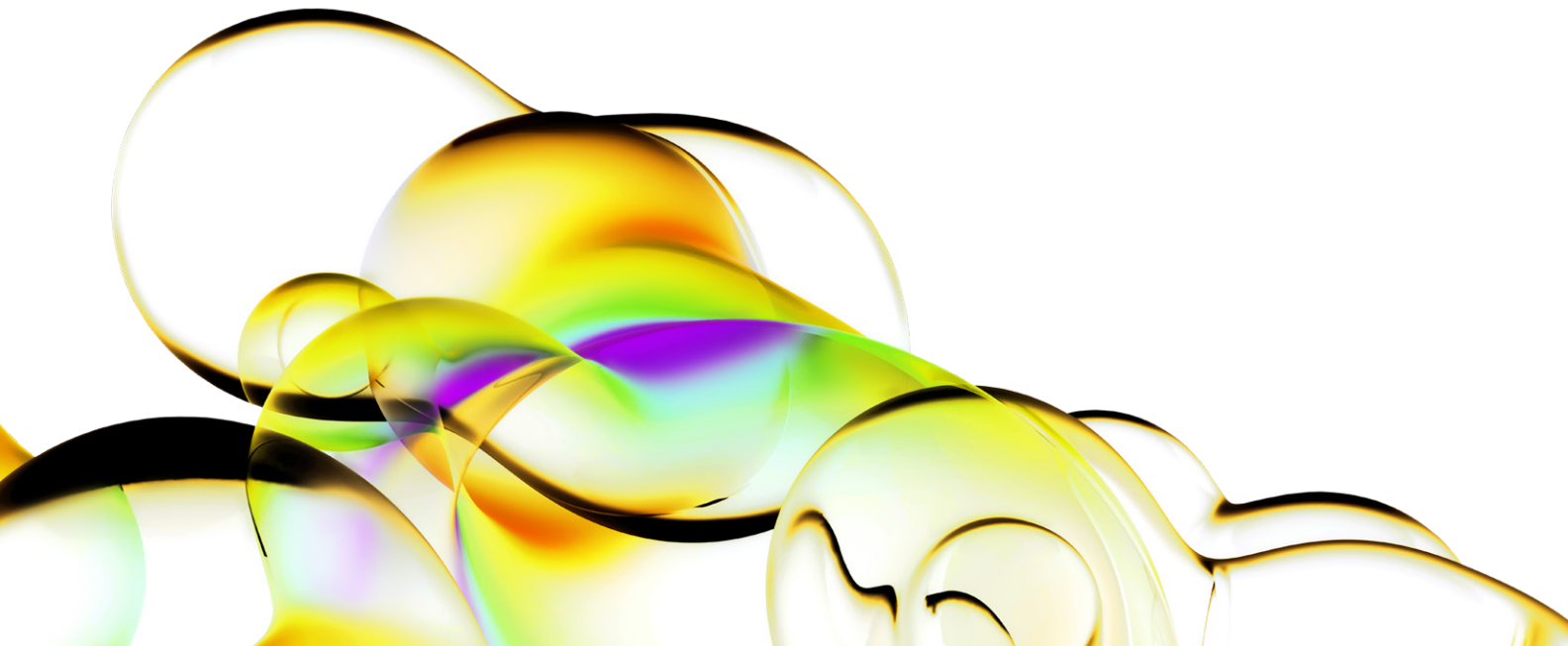
Introduction

Asthma is an inflammatory disease process characterized by reversible airway obstruction and airway hyperresponsiveness. This disease process is driven by activated T lymphocytes and eosinophils that are recruited to the lung upon inhalation of triggering allergens. These cells release inflammatory mediators, activate mast cells and epithelial cells and stimulate mucus secretion, ultimately leading to airway obstruction. The incidence and severity of asthma is increasing worldwide, elevating the need for clinically relevant *in vivo* animal models that can be used to improve the understanding of asthma biology and the development of effective therapeutics. Here we illustrate the use of fluorescence tomography in combination with IVISense™ Pan Cathepsin 680 probe, a near-infrared, protease-activatable imaging agent, for the noninvasive *in vivo* imaging and quantitation of pulmonary inflammation.

Noninvasive quantitative tomography of mouse asthma

Asthma can be induced in female BALB/c mice by provoking specific immunity to ovalbumin in the lung.[1] To do this, we immunized 6- to 8-week-old BALB/c mice on day 0 and day 14 by intraperitoneal injections of 50 µg of ovalbumin combined with 2 g of aluminum hydroxide as an adjuvant to enhance the allergic response. Between day 21 and day 24, mice received daily intranasal challenges with 100 µg of ovalbumin solubilized in PBS (pH 7.4) to focus an ovalbumin-specific



allergic response within the airways. The allergic response to ovalbumin induces cytokines and immune factors typical of those in human asthma (for example, interleukin (IL)-4, histamine and IgE), a large influx of eosinophils and changes in airway hyperreactivity. Measurement of these parameters requires surgical procedures (for airway hyper-reactivity), assessment of sacrificed mice (for bronchoalveolar lavage (BAL) eosinophil count) and extensive sample handling and preparation (for serum and BAL fluid microplate assays).[2]

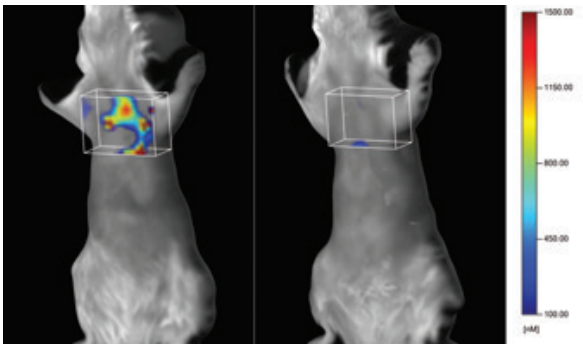


Figure 1: Noninvasive tomographic imaging of disease-associated protease activity in ovalbumin-induced mouse asthma. The asthmatic mouse (left) shows broad distribution of protease-activated IVISense Pan Cathepsin agent fluorescence in the lung, with little fluorescence seen in the lung region of a control mouse (right). The box marks the region of interest established for analysis.

Fluorescence molecular tomography offers a noninvasive alternative to invasive and terminal assessments of mouse pulmonary inflammation.[3] Fluorescence molecular tomography provides a rich dataset to mathematically model and reconstruct tomographic quantitative distribution of all sources of fluorescence within the subject. Pairing this technology with Revvity's IVISense Pan Cathepsin 680 near-infrared fluorescence agent allows the accurate detection and quantitation of protease activity associated with eosinophils;[4] the IVISense Pan Cathepsin probe is optically silent and fluoresces only when activated by disease-related proteases such as cathepsins. The near-infrared fluorochrome allows maximal tissue penetration and minimal absorption by physiological absorbers such as hemoglobin or water.

To quantitate the inflammation associated with asthma progression, we injected the mice with 5 nmol of IVISense Pan Cathepsin 680 agent at day 24, 4 hours after the final intranasal administration of ovalbumin; that is, 24 hours before imaging. On the day of imaging, we anesthetized the mice and positioned them in the imaging chamber of the optical imaging system. This system provides both tomographic (for deep-tissue fluorescence) and two-dimensional fluorescence reflectance imaging (for skin fluorescence and assessment of excised organs and tissues). Tomographic imaging of asthmatic mice yielded clear fluorescence signal in lungs of asthmatic mice, but not in control mice that received PBS instead of ovalbumin intranasal challenge (Figure 1). Analysis of the tomographic imaging data sets measured a highly significant increase in activated IVISense Pan Cathepsin agent fluorescence in the lung (>70 pmol/lung) as compared to <5 pmol in the lungs of control mice (P = 0.002; Figure 2a). These results correlate well with the eosinophil count from BAL samples (Figure 2b).

Asthmatic lungs removed from euthanized mice showed widespread fluorescence (Figure 3a), with minimal fluorescent background evident in control tissue. Measurement of the ratios of fluorescence intensity in lungs of mice with induced asthma yielded signal intensity >3.5-fold higher than that in lungs of control mice (Figure 3b). Notably, the individual fluorescence ratios for each excised lung correlated well with the noninvasive quantitation performed by the fluorescence tomography system (Figure 3c), supporting the contention that tomographic assessment was solely quantitating lung fluorescence. This confirms that the noninvasive tomographic assessment of asthmatic mice can be used to accurately assess deep tissue changes in protease activity associated with asthma, in agreement with BAL eosinophil counts (Figure 2b).

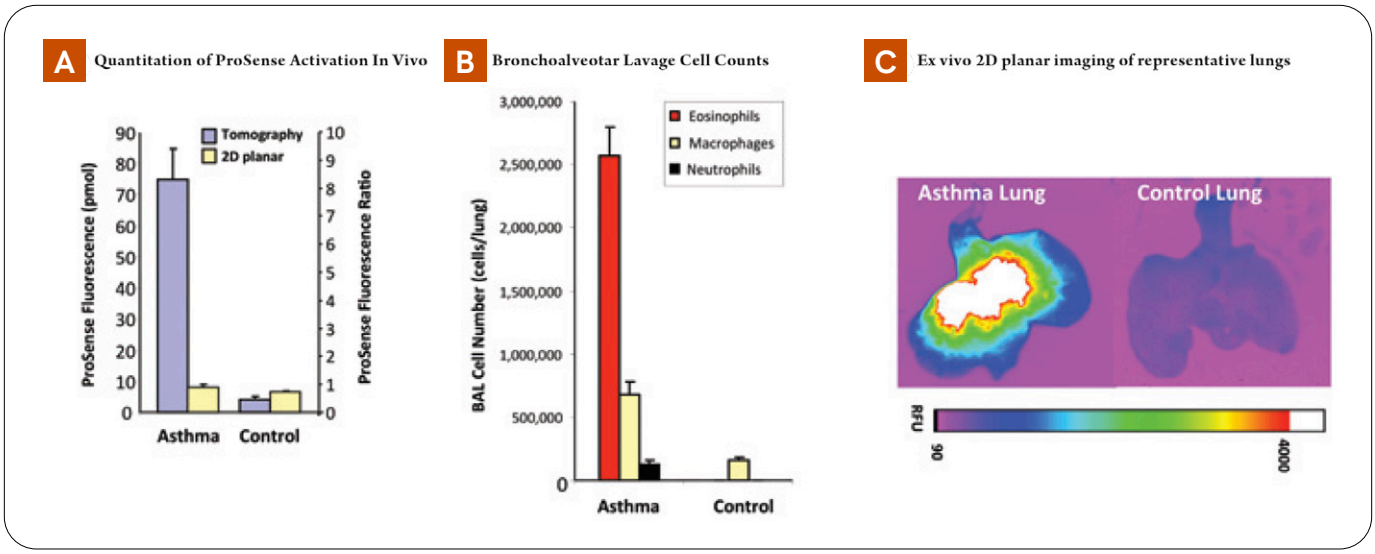


Figure 2: Assessment of asthma severity using quantitative tomography and bronchoalveolar lavage cell counts (terminal). **(A)** Quantitation of tomographic images shows high amounts of IVISense Pan Cathepsin 680 fluorescence within the lung region of asthmatic mice, with minimal fluorescence measured within that of control mice. Whole body 2D planar imaging detected no differences between asthma and control mice. Error bars, ± s.e.m. **(B)** BAL assessment indicates large numbers of eosinophils infiltrating the lungs of asthmatic mice compared to those of control mice, consistent with *in vivo* imaging results. The experiment is representative of five separate studies; n = 5 mice per group. Error bars, ± s.e.m. **(C)** Non-quantitative 2D planar imaging of representative lungs excised from asthmatic and control mice is consistent with *in vivo* imaging results.

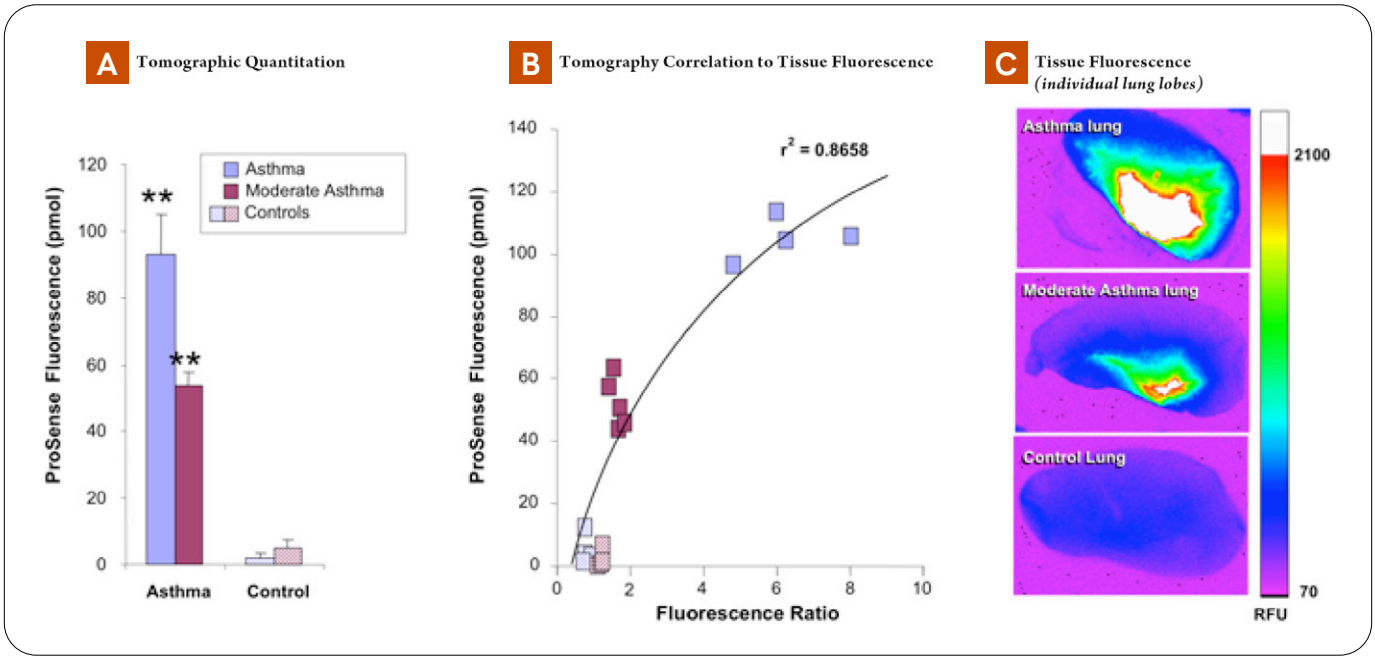


Figure 3: *Ex vivo* correlation between noninvasive quantitative tomography and *ex vivo* tissue assessment. **(A)** Tomographic quantitation shows clear quantitative differences between cohorts of mice with differing levels of disease. The results shown are representative of five separate studies; n = 5 mice per group. **(B)** Comparison of non-invasive imaging assessment and *ex vivo* tissue assessment of asthma yields a good correlation between the two readouts. Fluorescence molecular tomography yields greater sensitivity in discriminating between full asthma, moderate asthma, and controls, whereas *ex vivo* tissue assessment (ratio to mean control tissue fluorescence) was relatively insensitive at detecting moderate asthma relative to negative controls, yet detected significant differences between moderate and full asthma. **(C)** Excised lung tissues (separated into individual lung lobes) provided images revealing significant asthma-related increases in lung fluorescence intensity and distribution patterns differing with asthma severity.

Conclusion

These studies demonstrate the utility of fluorescence molecular tomography in combination with a near-infrared, protease-activatable fluorescence agent (IVISense Pan Cathepsin 680), for the noninvasive *in vivo* imaging and quantitation of pulmonary inflammation. After inducing asthma by ovalbumin in BALB/c mice, we effectively imaged the IVISense Pan Cathepsin 680 agent to detect eosinophil protease signal that can be tomographically quantitated using fluorescence tomography. Correlation of noninvasive tomographic quantitation with invasive assessment of lung inflammation established the accuracy of fluorescence tomography quantitation of deep tissue inflammation. Fluorescence molecular tomography also offers the potential for repeated noninvasive imaging and multiplex detection of multiple biological activities, allowing researchers to better study the progression and alteration of processes associated with disease progression and therapeutic intervention.

References

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