Validation of B-variant Ciz1 as a circulating biomarker for lung cancer

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Introduction
Ciz1 is a nuclear matrix protein (1) that promotes initiation of mammalian DNA replication (2) and tumourigenesis in mice (3). Ciz1 exists as multiple transcript variants, some of which have been implicated in disease [4,5,6]. All appear to be primarily nuclear, but have different sub-nuclear patterns and in some cases compromised attachment to the nuclear matrix. Variant isoforms in which the nuclear matrix attachment is disrupted are common in cancer cells. Here we describe a molecular abnormality in Ciz1 that is restricted to tumour cells making exploitation tractable. B-variant Ciz1 is prevalent in lung cancer cells, and evident in tumour biopsies but not in adjacent tissue, giving rise to a stable protein that can be robustly detected in plasma from patients with lung cancer (7).

Methods
Using a variant-selective antibody we quantified b-variant protein levels in two independent sample sets, with 170 (not shown) and 160 patients (results), by western blot. Band intensities were quantified by densitometry, normalised to endogenous immunoglobulin level, and expressed relative to constant calibrator samples. Examples of raw data generated from 0.5 μl of plasma from 20 individuals with stage 1 squamous cell carcinoma and 20 control samples from individuals with a smoking history but no diagnosed disease are shown below.

Results: b-variant in plasma

Variant Ciz1 protein in 160 plasma samples. A) Box plot showing results for 80 smokers with more than 10 years of smoking history and 40 stage 1 NSCLC patients with similar history (left), and 20 individuals diagnosed with stage 1 adenocarcinoma, inflammatory lung disease (granuloma), benign lung nodules (carcinoid, hamartoma), or stage 1 squamous cell cancer (right). B) ROC curves with 95% confidence intervals, for the indicated comparisons. Students twa-tailed t-test with unequal variance returned a p-value of <0.0001 for the cancer samples compared to either smokers, or benign and inflammatory disease.

Results: assessment of new b-variant antibody

New antibody (043) does not detect control band that the original antibody (2B) does but is more selective for b-variant. A) Western blot of samples probed with 043. B) Box plots showing the relative levels of b-variant Ciz1 in the normal and cancer samples for the same sample set probed with 2B and 043 compared to the control band with 2B, as indicated. C) ROC analysis with 95% confidence intervals for the indicated comparisons showing excellent discriminatory capability.

Results: b-variant Ciz1 stability

A time course analysis of plasma incubated at 37°C showing b-variant and normalised b-variant levels. Three samples were analysed as described in the methods section, error bars represent standard error of three technical replicates.

Conclusions:
Variant Ciz1 is
• a highly stable tumour antigen
• present in plasma but not serum
• easily detected in less than a μl of plasma from lung cancer patients
• evident in patients with stage 1 lung cancer
• capable of discriminating between stage 1 lung cancer and benign lung nodules

In the context of early detection, application to patients with CT positive nodules could halve the number subjected to unnecessary procedures.

Clinical development:
Some of these results have been published2 and a commercial development partnership has begun between Fujirebio Diagnostics Incorporated and Cizzle Biotech. This will co-develop an ELISA test for the detection of early stage lung cancers that can be taken forward through regulatory and clinical development worldwide.