## PART VI

## TECHNICAL REPORT

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The Directors and the Proposed Directors
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Dear Sirs,

**RE: Technical report on Cizzle Biotechnology Limited ("Cizzle Biotechnology" or the "Company")**

Founded over 20 years ago, Hardman & Co has built a reputation for providing a broad range of advisory and consulting services. These include independent research of the highest quality, valuations, and due diligence assessments. Hardman & Co has been involved in "Expert Opinion and Valuation" work in legal cases, and is increasingly being asked for independent valuation services on unquoted companies to satisfy the focus of the Financial Conduct Authority (FCA Discussion Paper DP17/1 – February 2017) on the valuation of unquoted/illiquid assets in the daily pricing of funds, which may require fund administrators to verify the private valuations provided by portfolio managers. Our services are provided by a team of highly skilled and qualified industry professionals. Most of our analysts have been recruited from many of the leading investment houses and cover most major equity sectors, with Life Sciences (encompassing pharmaceuticals, biotechnology, medtech and diagnostics) being a particular strength.

This report has been prepared by the life sciences research team at Hardman & Co, comprising:

Dr Martin Hall, B.Pharm.S (Hons), MRPharmS, Ph.D

Hardman & Co has prepared this report for the directors and the proposed directors of Bould Opportunities plc ("Bould") and for Bould's financial adviser, Allenby Capital Limited, for inclusion in the prospectus being issued in relation to the placing on new ordinary shares and the proposed acquisition of Cizzle Biotechnology by Bould, prior to the admission of Bould's entire issued and to-be-issued ordinary share capital to the Official List (by way of Standard Listing under Chapter 14 of the Listing Rules) and to trading on the London Stock Exchange's main market for listed securities (the "Prospectus").

### 1. Purpose of this report

This report considers the business opportunity for Cizzle Biotechnology within the global environment for the clinical diagnosis of lung cancer. Within this framework, the technical competencies of Cizzle Biotechnology are analysed against its peers and concludes with consideration of the overall opportunity and competitive advantage for Cizzle Biotechnology in an intensely-competitive and rapidly-growing global cancer diagnosis market.

Hardman & Co declares that it is responsible for this report, which forms part of the Prospectus, and that, to the best of Hardman & Co's knowledge, the information contained in this report is in accordance with the facts and this report makes no omission likely to affect their import. To the fullest extent permitted by law,

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## 2. Methodology

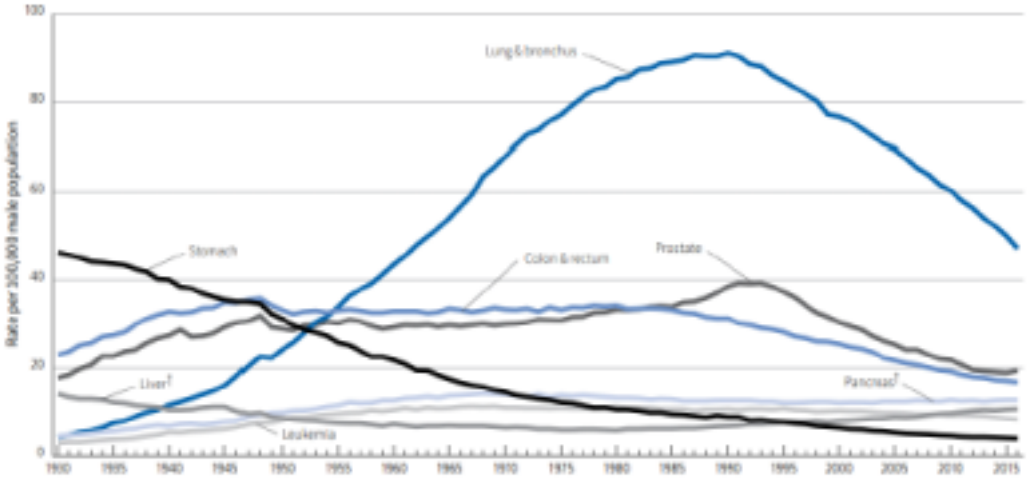
The Life Sciences team at Hardman & Co has reviewed relevant documentation and presentations provided by Cizzle Biotechnology and interviewed the Founder and Science Director of the Cizzle Biotechnology. These sources were supplemented by publications in peer-reviewed scientific journals, information in the public domain, the team's internal information database and our extensive experience in the pharmaceutical and healthcare industries. We have used all due care in ensuring the accuracy and completeness of the information and data presented but developments in these industry areas occur rapidly, which could result in a change in clinical practice, and render some or all of the information or conclusions incomplete, obsolete or invalid.

## 3. Diagnosis of lung cancer

### *Setting the scene*

Lung cancer is the second most common cancer in both men and women in the US[1] and the leading cause of cancer death worldwide[2]. Despite a downward trend in the US death rate from lung cancer, it still accounts for one-in-five of all deaths caused by cancer.

**Figure 1: Trends in age-adjusted cancer death rates* by organ in US males 1930-2016**




**Per 100,000, age adjusted to the 2000 US standard population. †Mortality rates for cancer of the pancreas and liver cancers are increasing.*

*Source: US Mortality Volumes 1930 to 1959, US Mortality Data 1960 to 2016, National Center for Health Statistics, Centers for Disease Control and Prevention[1]*

1 American Cancer Society – US statistics 2019
2 American Cancer Society – Global statistics 2018

The American Cancer Society estimates that there were 228,150 new cases of lung cancer (116,440 in men and 111,710 in women) in the US in 2019 and 142,670 deaths (76,650 in men and 66,020 in women). By far the most important risk factor for lung cancer is smoking, which accounts for ca.85 per cent. of all US lung cancer cases and results in ca.81 per cent. of lung cancer deaths[1]. Although the prevalence of smoking has decreased, approximately 37 per cent. of US adults are current or former smokers[3]. The incidence of lung cancer increases with age and most commonly occurs in persons aged 55 years or older. Therefore, increasing age and cumulative exposure to tobacco smoke are the two most common risk factors for this disease.

In China, lung cancer is the most common cancer and the leading cause of cancer death[4]. Along with socio-economic development, environmental problems have intensified and the burden of lung cancer continues to increase. Unlike the situation in many countries, cigarette consumption in China continues to rise. As the most densely-populated country in the world, China contains 19 per cent. of the global population with 21.8 per cent. of all newly-diagnosed cancer cases and 26.9 per cent. of deaths, including 35.8 per cent. of all newly-diagnosed lung cancer cases and 37.6 per cent. of lung cancer deaths worldwide[4].

### *Types of lung cancer*

There are three different types of lung cancer:

- **Non-small cell lung cancer (NSCLC):** This is the most common type of lung cancer comprising about 85 per cent. of all cases. NSCLC is itself divided into three subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma. The five-year survival rate differs with stage at diagnosis, and is ca.60 per cent. localised, 33 per cent. spread to surrounding tissues and 6 per cent. spread to other organs.
- **Small cell lung cancer (SCLC):** This represents 10 per cent.-15 per cent. of cases, and is characterised by its rapid spread to other organs, which is reflected in the low five-year survival rates: ca.29 per cent. localised, 15 per cent. surrounding tissues and 3 per cent. spread.
- **Lung carcinoid tumour (LCT):** This accounts for less than 5 per cent. of all cases. The tumours are sometimes referred to as lung neuroendocrine tumours; they are characterised by being slow-growing and rarely spread to other organs. The five-year survival rate is 97 per cent. localised, 87 per cent. surrounding tissues and 57 per cent. spread.

### *Stage of diagnosis*

In its early phase, lung cancer usually develops without any obvious symptoms and is difficult to detect with traditional radiographic methods, even to the trained eye. Even when there are symptoms, many people may mistake them for other problems such as a respiratory infection or simply the consequence of the long-term effects of smoking. In addition, lung cancer is affected by stigma, whereby patients feel guilty about their smoking history and lifestyle, and delay talking to their doctor about potential symptoms[5].

Lung cancer is divided into four stages, with stage I being localised to the lung through to stage IV where the cancer has metastasised into distant organs. Given the late detection and the complex optimal lung cancer care pathway (see Figure 4), the overall prognosis is poor, which explains why it is one of the leading causes of death. Data for both the UK[6] and US[7] are broadly similar, with lung cancer patients most commonly diagnosed at stage IV. About three quarters of patients are diagnosed at a late stage (72 per cent.-76 per cent. are diagnosed at stage III or IV), whereas about one quarter are diagnosed at an early stage (24 per cent.-28 per cent. are diagnosed at stage I or II). Therefore, most people are diagnosed at a stage where prognosis is poor, as evidenced by the US five-year survival data.

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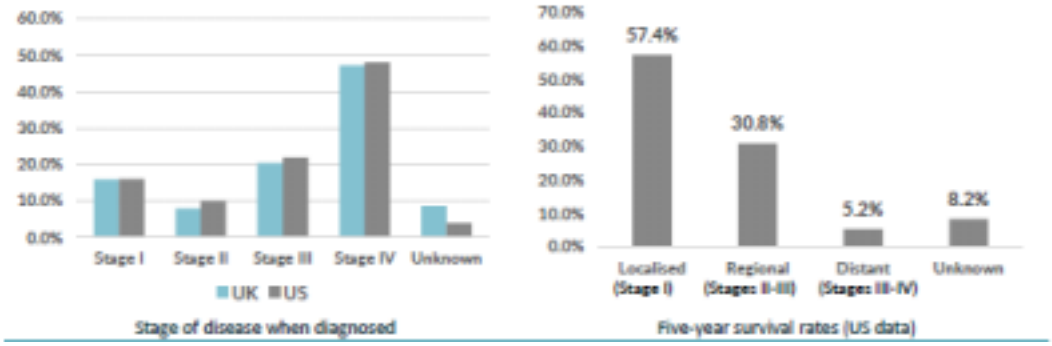
3 US Preventive Services Task Force
4 Chen et al., 2015
5 Global Lung Cancer Coalition
6 Cancer Research UK
7 US National Cancer Institute

**Figure 2: Lung cancer: stage of diagnosis and five-year survival rates**




*Source: Cancer Research UK[5], US National Cancer Institute[6], Hardman & Co Life Sciences Research*

The Lung Ambition Alliance highlighted a recent survey[3] conducted by the Global Lung Cancer Coalition (GLCC) to coincide with the end of National Lung Cancer Awareness Month, which showed that 87 per cent. of people were in favour of implementing a national program in their country to increase the detection of lung cancer in the early stages. However, despite screening procedures for diagnosis of lung cancer being available, many countries have not yet adopted them, despite evidence suggesting that lung cancer screening saves lives[8]. The GLCC concluded that "…the time is ripe to consider diagnostic testing as a key priority for those at risk of lung cancer and favours the adoption of guidelines that can help increase screening rates…"

***Symptoms***

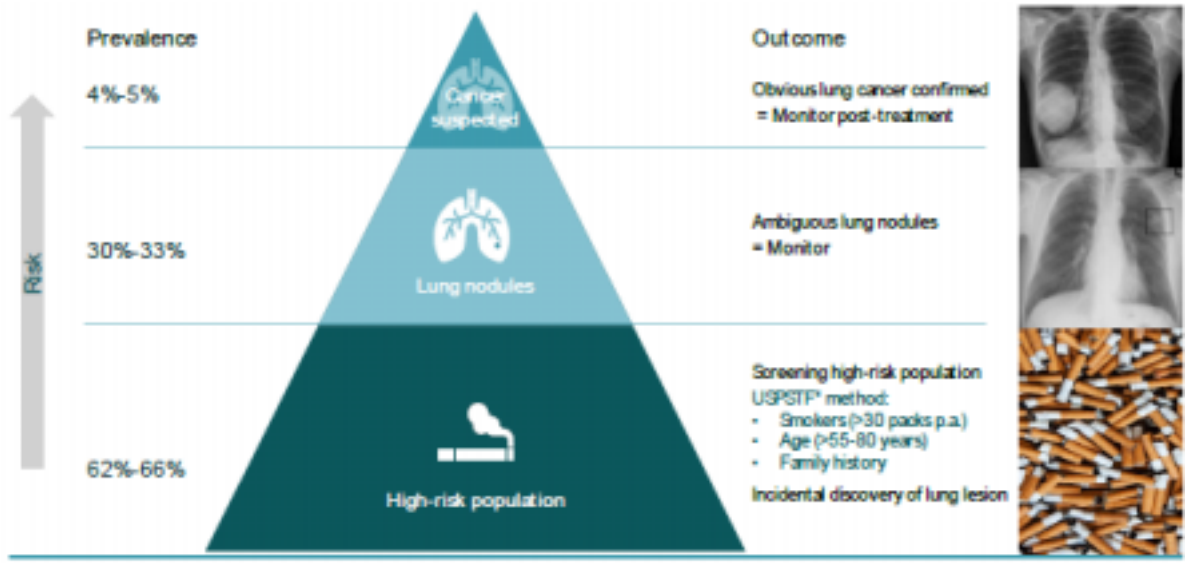
Diagnosis of lung cancer is an extremely complex process. Patients usually present to their general practitioner (GP) with one or more of the following symptoms:

- persistent cough;
- weight loss;
- coughing-up blood;
- chest pain;
- chest infection that has failed to resolve.

The GP will also take into account the patient's age and history of smoking. Based on this information, if a patient is considered to be high risk, the GP is likely to refer him/her to the chest clinic at the local hospital, which would trigger a complex treatment pathway[9,10]. About 70 per cent. of lung cancer patients are identified via this route. The other 30 per cent. are identified in the hospital setting via "incidental findings", whereby a patient attends A&E having already seen his/her GP and the problem persists, or the patient attends hospital for a completely different reason and something suspicious is found on a scan – usually a chest X-ray (CXR) or a chest CT.

8 Yousaf-Khan U, et al., 2017
9 National Optimal Lung Cancer Pathway

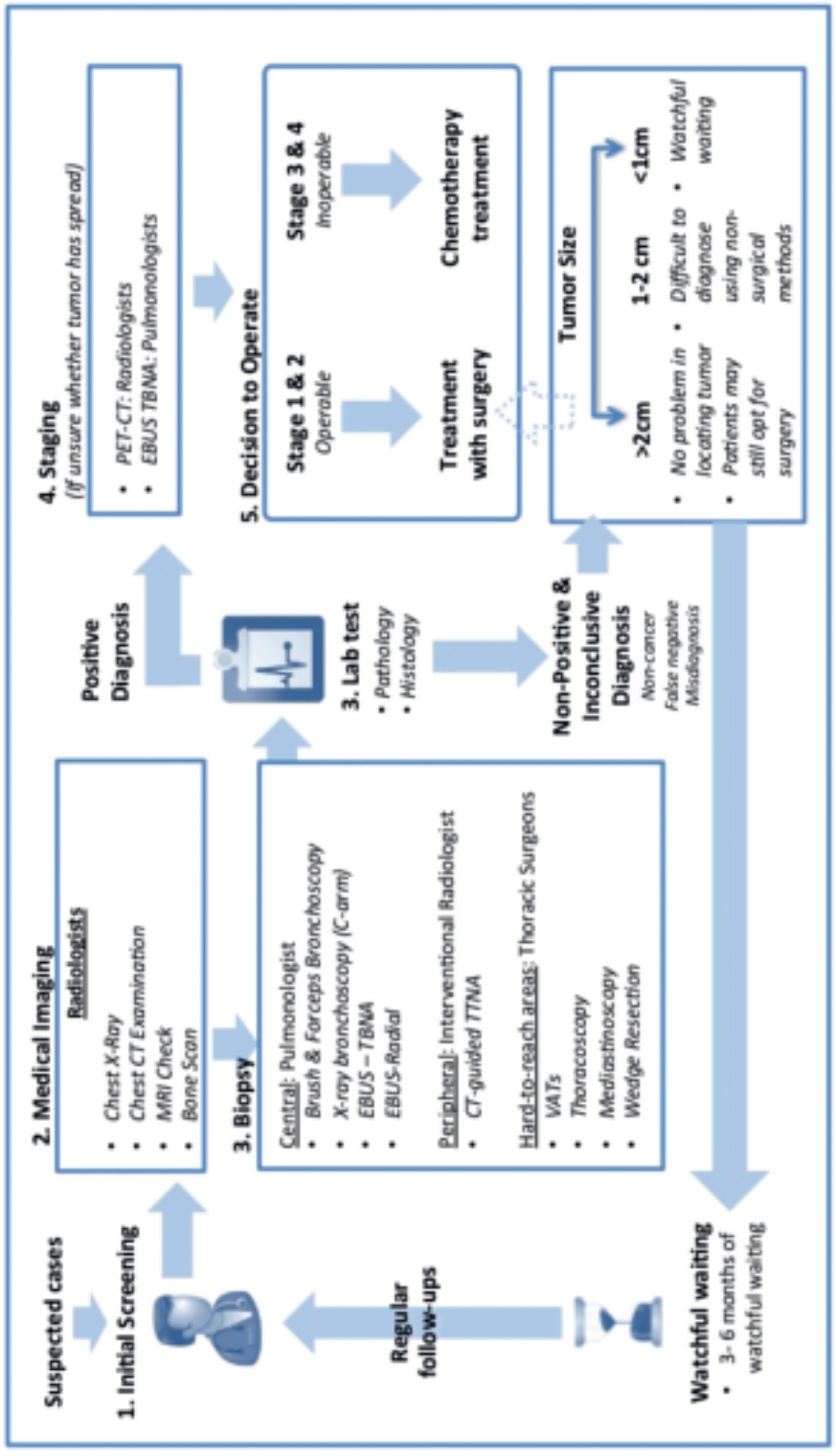
**Figure 3: Lung cancer diagnosis – key populations**




*Sources: Case courtesy of A.Prof Frank Gaillard, Radiopaedia.org, rID: 10561, Beschreibung: Konventionelles Röntgenbild des Thorax (der Lunge) mit rundlicher Verdichtung in der linken Lunge Quelle: selbst erstellt —de:Benutzer:Lange123 17:18, 11. Nov. 2004 (CEST), France3-regios (PROVENCE-ALPES-CÔTE D'AZURBOUCHES-DU-RHÔNE), *US Preventive Services Task Force, cancer.org, Hardman & Co Life Sciences Research*

Even after a positive scan, the patient is classified as "high clinical suspicion" requiring further tests. What happens next depends on local protocol, despite there being "National Optimal Lung Cancer Pathway" guidelines in place in many countries. However, it will usually involve either another more detailed scan or a tissue biopsy, both of which can be upsetting for the patient and costly to the healthcare system.

**Figure 4: Typical lung cancer care pathway**




*Source: https://www.slideshare.net/edenstrategyinstitute/asias-quiet-war-on-lung-cancer* [10]

Because it is simpler, most suspected lung cancer patients will undergo another scan. However, it is estimated that 90 per cent. of people having a confirmatory scan due to the presence of a size-qualifying nodule do not actually have lung cancer. Also, for people that have CXR or CT scans for other reasons, about 13 per cent. of these have a size-qualifying nodule but 98 per cent. do not have cancer. Furthermore, all of these cases will be monitored for up to two years, with chest CT scans every six months. This

[10] www.slideshare.net

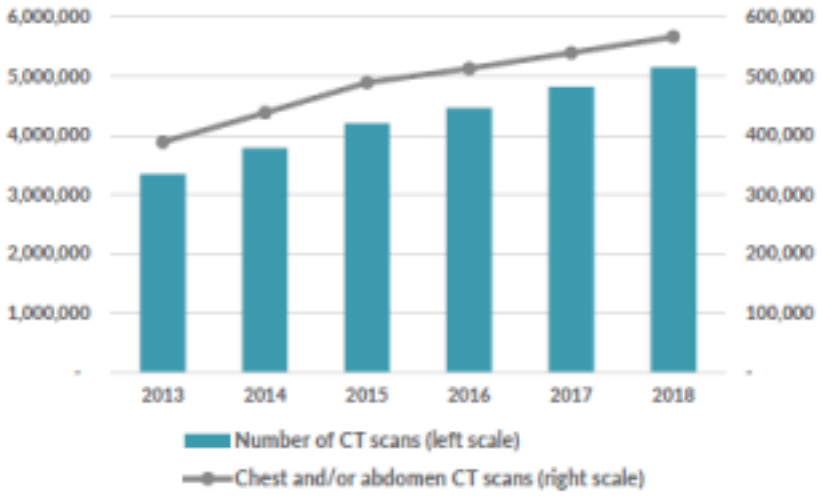
represents a huge burden and cost for the healthcare system, unnecessary overloading in lung cancer clinics and, importantly, is upsetting for patients who do not have cancer.

At the current time, tissue biopsy remains the standard-of-care to confirm the initial diagnosis, which allows pathologists to analyse complete cells within tumours. While tissue biopsy of cancerous tissues is essential in determining the type of cancer and guiding the immediate treatment regime, there is a strong need for early and accurate patient triage – i.e. is the nodule seen on the CXR or CT scan cancerous or not.

***Clinical burden***

A more efficient screening test that minimises false-positive interpretations would circumvent the misdiagnosis and late detection of lung cancer. Usually, patients with size-qualifying nodules would be followed up for two years via a chest CT scan every six months, with each one taking ca.30 minutes to perform (10 minutes for preparation + 15-20 minutes for the test). To put this in perspective, the number of CT scans performed in the NHS in the 12 months ending March 2019 was around 5.15m[11], with a five-year CAGR growth at 6.9 per cent.. Approximately 11 per cent. of these scans were for the chest and/or abdomen. Owing to increased pressure on the scanners (and staff), the time taken from the date of request to the date of test averaged 16 days.

**Figure 5: CT scans performed in the UK, 2013-18**




*Source: NHS England*

Use of a suitable companion diagnostic tool/biomarker alongside the CT scan to confirm/refute the malignant character of suspicious nodules would be beneficial for both the payors and patients.

## 4. Cizzle Biotechnology Limited

The Mammalian Cell Cycle Research Group, in the Department of Biology at the University of York, under the leadership of Professor Dawn Coverley, is a global expert in the research of Cdkn1A-interacting zinc finger protein 1 (Ciz1), a naturally-occurring cell nuclear protein that promotes DNA replication. Outside of this group, the volume of research on this protein is relatively limited. Largely through grant funding, this academic group discovered that Ciz1 is altered in a number of common cancers. Cizzle Biotechnology was established (incorporated in England and Wales with company registration number 5249093) to enhance the understanding of the variants of Ciz1 and to develop and commercialise diagnostic cancer tests.

[11] NHS England – Diagnostic Imaging dataset

**Table1: Integrating academic and private research**

| *Mammalian Cell Cycle Research Group, University of York* | *Cizzle Biotechnology Ltd* |
|---|---|
| Grant-funded academic research | Investment-funded research |
| ● Function of Ciz1 in normal cells | ● Expression of Ciz1 variants in cancer |
| ● Biological context | ● Variant Ciz1B |
| ● Profiling Ciz1 variants | ● Diagnostic tests based on Ciz1B |

*Source: Cizzle Biotechnology, Hardman & Co Life Sciences Research*

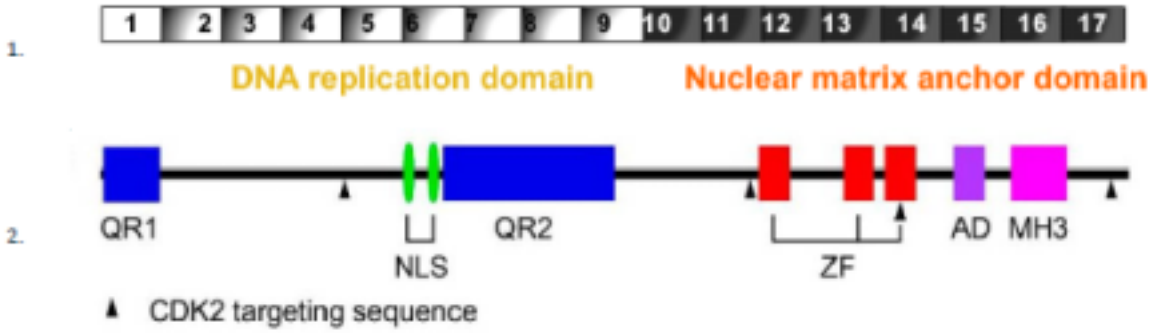
Cizzle Biotechnology is now ready to move to the next stage of development, converting its proof-of-principle prototype test to a commercial monoclonal antibody-based test for the accurate diagnosis of early-stage lung cancer.

## 5. Elucidation of Ciz1 protein

### *Background*

Ciz1 is a naturally occurring protein that was first described in 1999[12] and consists of an 898 amino acid residue chain in humans. Relatively few research laboratories have investigated the Ciz1 protein, with much of the academic work to elucidate its normal function and its potential role in tumorigenesis (promoting cancer) being undertaken by the team of Professor Dawn Coverley, at the University of York.

**Figure 6: Simplistic schematics of Ciz1 protein**




*Source: 1. Cizzle Biotechnology, 2. Liu et al[13]*

- **Glutamine-rich domains (QR1 and QR2)** are not yet related to Ciz1's role in DNA replication. Abnormal expansion may lead to misfolding and aggregation of neurodegeneration-related proteins.
- One of the main functions of **Zinc-finger motifs (ZF)** is to bind nucleic acids.
- Various studies have shown that the **acidic domain (AD)** is associated with a protein's stability and its ability to interact.
- The **MH3 domain** is found in matrin 3, a nuclear matrix protein, and NP220, a DNA-binding nuclear protein, suggesting that Ciz1 may bind to DNA or nuclear matrix-associated RNA.

### *Role in DNA replication*

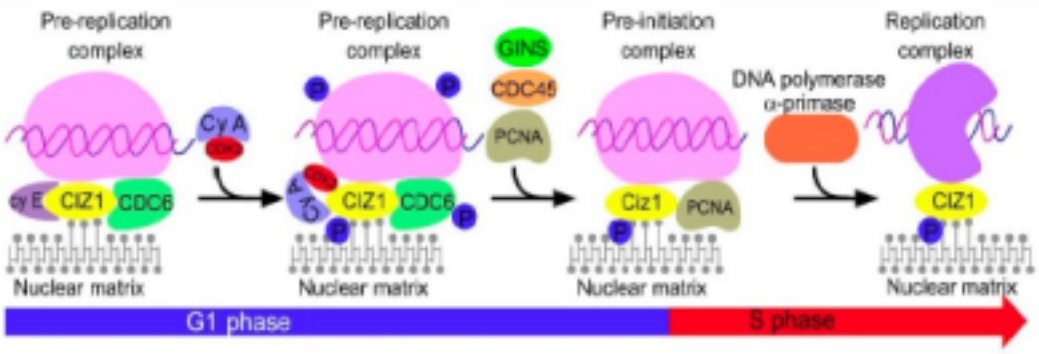
Ciz1 is a component of the cell nucleus and has been shown to play a role in DNA replication and cell cycle regulation. Ciz1 interacts with several proteins that contribute to the regulation of cellular proliferation (including transcriptional regulators), cell cycle regulators (including, among others, cyclin E, cyclin A and CDK2), and proteins that are not directly related to DNA replication[14]. Consequently, Ciz1 is considered to be involved in numerous biological functions.

12 Mitsui et al., 1999
13 Liu et al., 2016
14 Pauzaite et al., 2016

Various experiments have been performed that support the hypothesis that Ciz1 plays a role in DNA replication:

- in both cell-free and cell-based experiments DNA replication can be stimulated by recombinant Ciz1; and
- the lack of Ciz1 has been shown to delay replication of DNA[15].

**Figure 7: Role of Ciz1 in DNA replication**




*Source: Liu et al*[13]

### *Function of Ciz1*

A prerequisite for the health and longevity of multicellular organisms is the precise duplication of the genome. In order for this to occur, regulation of DNA replication is required prior to the genome segregating into daughter cells. This process is regulated at multiple levels to ensure near-perfect chromosome duplication with error rates at less than one per billion bases copied[16]. This level of precision requires highly-orchestrated and stratified mechanisms to ensure that DNA replication occurs once, and only once, per cell cycle. Crucially, the proteins that are associated with DNA and the chemical modifications that they bear must also be copied accurately. When something goes wrong in this complex process, biological dysfunction results.

Through deletion, overexpression or alternative splicing, Ciz1 is associated with tumour growth in SCLC and NSCLC, colorectal, breast, prostate, hepatocellular carcinoma and gall bladder cancer, and lymphoma and leukaemia. In each case, there is a cancer-specific alteration resulting in loss of or increased Ciz1 protein levels or alternative splicing of the Ciz1 transcript.

**Table 2: Ciz1 associations in multiple cancers**

| *Cancer type* | *Ciz1 alteration* | *Result of intervention* |
|---|---|---|
| Lung | Alternative splicing – Ciz1b | Reduced tumour growth in xenograft models |
| Colorectal | Overexpression | Reduced proliferation, and colony formation *in vitro* |
| Gall blabber | Overexpression | Reduced xenograft tumour growth<br>Reduced tumour migration *in vivo* |
| Prostate | Overexpression | Reduced tumorigenesis in xenograft models<br>Reduced G1 checkpoint activation |
| Breast | Overexpression | Increased oestrogen sensitivity and increased tumour size in xenograft models |
| Hepatocellular | Overexpression | Increased proliferation, migration<br>Primitive neuro ectodermal tumour |

*Source: Adapted from Pauzaite et al*[14]

15 Ainscough et al., 2007
16 Bebenek et al., 2004

### Ciz1 variants

Recently, a collection of mRNA variants of Ciz1 in humans, as a consequence of alternative splicing, has been defined, which has resulted in a significant loss of amino acid residues in different locations on the Ciz1 protein. Some of these have been shown to be disease specific. For example, variant Ciz1ΔE4, in which exon 4 is omitted, is found in Ewing's tumour cells. Another splicing form, variant Ciz1b, has been shown to be prevalent in lung tumours, and this is the subject of Cizzle Biotechnology's intellectual property (IP). Thus, alternative splicing of Ciz1 seems to affect the biological function of Ciz1 in various pathological processes.

**Table 3: Alternative splicing of Ciz1**

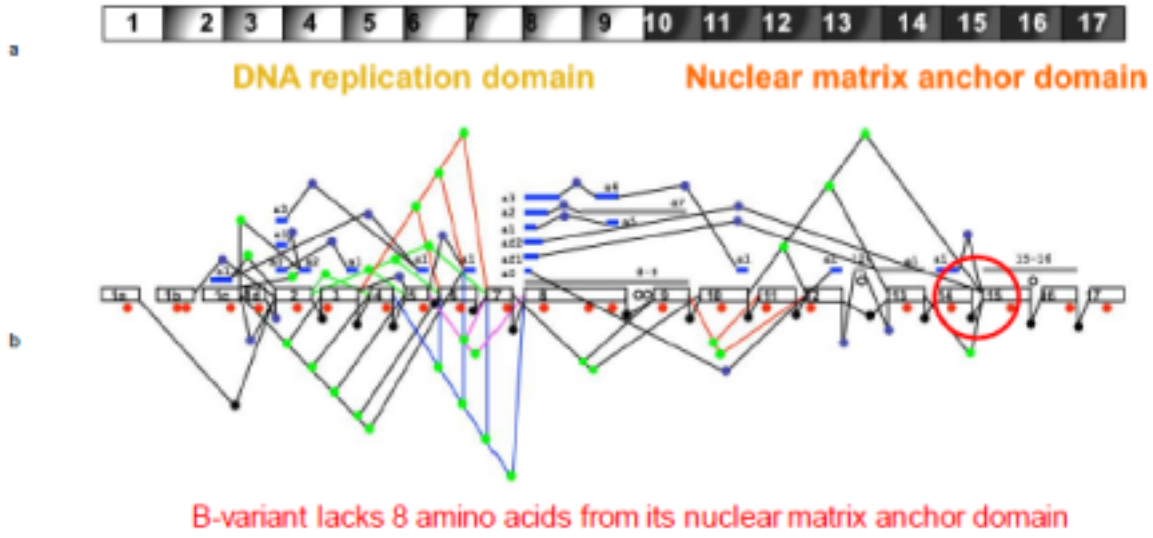
| *Ciz1 variants* | *Alternative splicing sites* | *Biological indication* |
|---|---|---|
| Ciz1ΔE4 | Exon 4 | Ewing's tumour |
| Ciz1S | Partial exon 8 | Alzheimer's disease |
| Ciz1M | Partial exon 8 | Alzheimer's disease |
| Ciz1ΔE8-12 | Exons 9,10,11; partial exons 8,12 | Ewing's tumour Primitive neuro ectodermal tumour |
| Ciz1b | Exon 14 | Lung cancer |

*Source: Adapted from Liu et al[13]*

### *Variant Ciz1b*

To date, the work of Cizzle Biotechnology has concentrated on the cancer-specific Ciz1b variant that lacks eight amino acids from its nuclear matrix anchor domain, and is implicated in lung cancer.

**Figure 8: Schematic to show Ciz1b variant**




*Source: Adapted from Cizzle Biotechnology (a) and Rahman et al[17](b) by Harman & Co Life Sciences Research*

### 6. Development of prototype Ciz1b diagnostic test

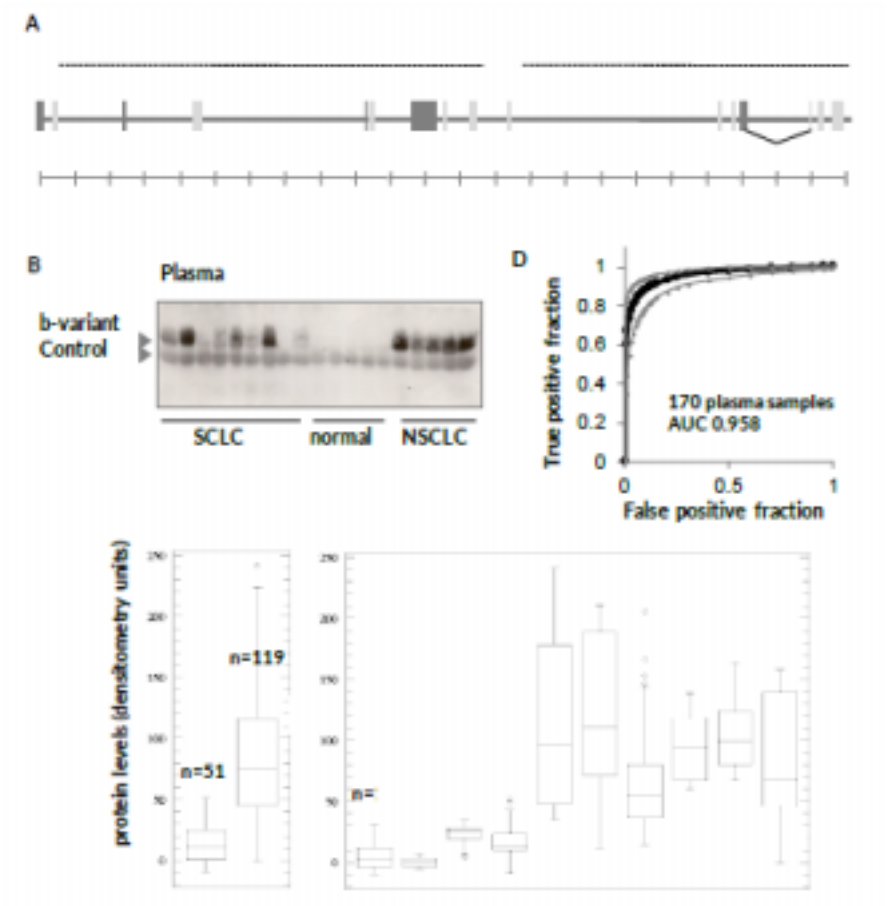
Cizzle Biotechnology has developed a quantitative immunoassay for measuring the Ciz1b biomarker in plasma taken from lung cancer patients[18]. The prototype test, based on a technique called Western blot (WB), has now been applied to 486 plasma samples derived from four independent sample sets including samples from patients with different types of lung cancer, asthma/COPD, and heavy smokers.

[17] Rahman et al., 2010
[18] Higgins et al., 2012

***Results from cohort 1***

Results from the measurement of variant Ciz1b protein in 170 samples in plasma set 1 were described in a peer-reviewed article published in the prestigious Proceedings of the National Academy of Science (PNAS)[17] and are reproduced in Figure 9.

**Figure 9: Test results from cohort 1**




*Source: Cizzle Biotechnology, Higgins et al[18]*

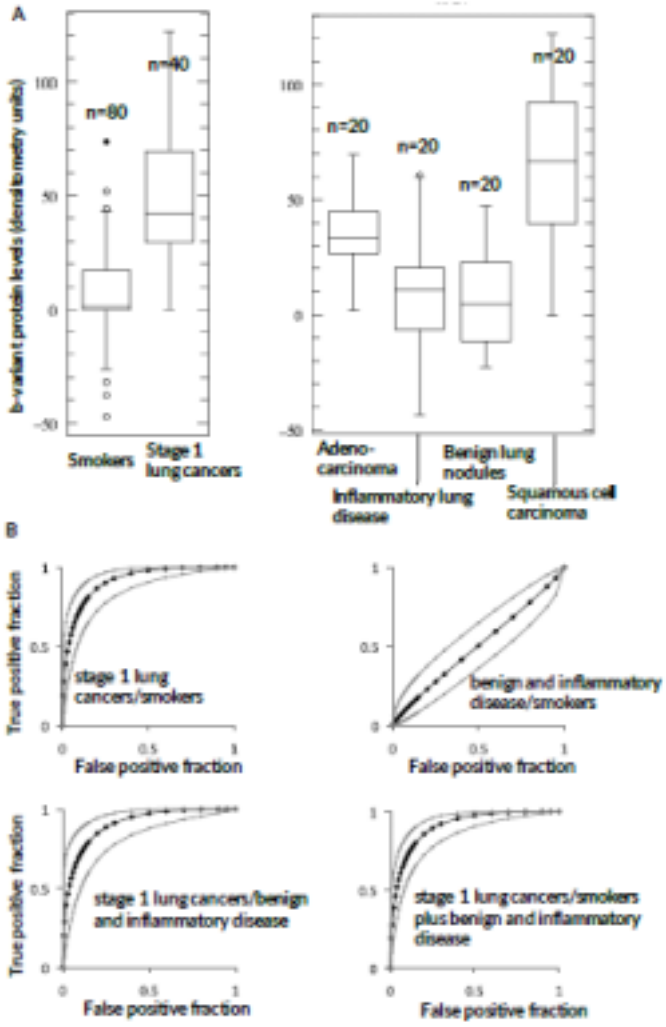
- **A:** Ciz1 gene showing translated exons (numbered). Exons that encode DNA replication domain (5) and nuclear matrix anchor domain (9) are indicated by dotted lines above.
- **B:** Summary of predicted and detected alternative splicing events, including exclusion of part of exon 14 to generate Ciz1B[16].
- **C:** Graphs showing the median-, upper-, and lower-quartile, range, and outliers for data derived from WB by densitometry. Results for a total of 119 pre-treatment lung cancer patients with the indicated type and stage of disease, plus 51 samples from individuals with no cancer disease buts with COPD, asthma, or anaemia. Using a threshold set at the mean of the noncancer samples, the test correctly classified 98 per cent. of all 119 lung cancer patients, with specificity of 85 per cent..

For cohort 1, when thresholds are high, so that 98 per cent.-100 per cent. of cancer patients are detected, the false positive rate is 45 per cent.. This is expected to be useful if applied after a chest CT to exclude CT-false positive patients. The sensitivity/false positive profile depends on where thresholds are set, and may differ with clinical context – for example pre-CT screening compared to post-CT validation.

***Results from cohort 2***

Results from the measurement of variant Ciz1b protein in 160 samples in plasma set 2 were presented in the same article[17] (Figure 10). The importance of this dataset is that each cancer group was closely aligned with a control set, and information on the patient histories and follow-up was very accurate. Crucially, the lung cancer patient samples were all known to reflect stage I disease.

**Figure 10: Test results from cohort 2**




*Source: Cizzle Biotechnology, Higgins et al[18]*

- A: Box plot showing results for 80 smokers with more than 10 years of smoking history and for 40 patients with stage I NSCLC with similar smoking history (left), and broken down into 20 individuals diagnosed with stage I adenocarcinoma, inflammatory lung disease (granuloma), benign lung nodules (carcinoid, hamartoma), or stage I squamous cell carcinoma (right), showing lower, median, and upper quartiles and outliers (circles).
- B: ROC curve with 95 per cent. confidence intervals for the indicated comparisons. AUCs are 0.913 when samples from 80 smokers are compared with samples from 40 patients with stage I lung cancer, 0.905 when samples from 40 patients with benign nodules or inflammatory disease are compared with samples from 40 patients with stage I lung cancer, and 0.909 when all samples from smokers and patients with benign nodules or inflammatory disease are compared with all samples from patients with stage I lung cancer. However, they are only 0.503 when samples from smokers are compared with samples from patients with benign nodules or inflammatory disease.

We understand that an important potential application is as a test for lung cancer in individuals with lung nodules identified by CT-scan. A test that could positively identify those individuals with stage I lung cancer could help reduce the frequency of surgical intervention and favourably affect both cost and outcome.

## 7. Development of a commercial test

The goal with any *in vitro* diagnostic test is to measure accurately the quantity of specific substances in an easily-sampled biofluid (blood/urine/saliva) in order to look for signs of disease or agents that cause disease, check for antibodies or tumour markers (biomarkers), and to see how well treatments are working.

The prototype diagnostic test using WB demonstrated that the Ciz1b biomarker could be measured with high sensitivity and a clinically useful false positive rate. However, such a study simply indicates that you have a demonstrable test that has the ability to select a positive outcome, but is less reliable when it comes to a high-throughput application in a hospital setting. Therefore, Cizzle Biotechnology needs to refine the test to generate one that would be suitable for commercial scale-up and kit manufacture.

### *What needs to be done?*

*Replacement of Western blot by ELISA-mAb*

First, use of the WB technique needs to be changed to a sandwich enzyme-linked immunosorbent assay (ELISA) linked to a monoclonal antibody (mAb) (or synthetic alternative), which is a more standardised procedure that would reduce the technical demand and high cost associated with WB, thus making it more acceptable from a commercial standpoint. This has already been achieved with a polyclonal antibody, and used to generate proof-of-concept data using a limited set of lung cancer patient samples[19].

Table 4: Comparison of Western Blot with ELISA

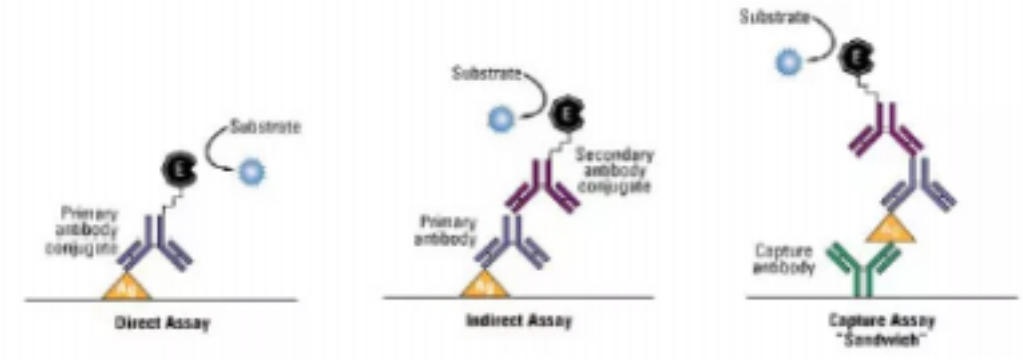
| *Characteristic* | *Western blot* | *ELISA* |
|---|---|---|
| Detection method | Immuno | Immuno |
| Sensitivity | High | High |
| Specificity | High | High |
| False positives | Potentially high | Potentially high |
| Quantification of specific protein | Can be poor | Good |
| Determine size of protein | Good | Very poor |
| Technical expertise needed | High level | Low level |
| Use in screening | Cumbersome | High throughput |

*Source: BioRad, Hardman & Co Life Sciences Research*

Typically, with an ELISA, a 96-well plate coated with one antibody is used, to which a sample is added and incubated for a period of time, before washing the plate a number of times to remove any unbound protein (that does not contain the specific antigen for which the antibody is geared). You then incubate with a second antibody that has a marker attached to it (usually an enzyme that gives a reaction that can be visualised with certain chemicals) and you do a second round of incubations and washes. Finally, you add the chemical that the enzyme reacts to and use a spectrophotometer to measure the colour change that is created in the individual wells of a 96-well plate.

There are three possible approaches to achieve this, but, based on preliminary work, the preferred approach is a sandwich assay with a mAb.

**Figure 11: ELISA – three possible approaches**




*Source: Adapted from Study Read: types of ELISA[20]; Hardman & Co Life Sciences Research*

[19] Coverley et al., 2017
[20] Study Read

The missing eight amino acids from the Ciz1b biomarker creates a unique junction against which an antibody can be formed. Cizzle Biotechnology knows that this is achievable having generated already two polyclonal antibodies with the desired specificity, but this needs to be replaced by a mAb, the "b-variant capture antibody". Development of a specific mAb would provide a renewable reagent with surety of supply.

Detection would be made by an anti-fibrinogen antibody – the detector – which could be an off-the-shelf purchase. The Ciz1b biomarker in patient's plasma samples naturally exists attached to fibrinogen in the blood, producing a complex that can be detected by sandwich ELISA. Preliminary work showing that fibrinogen can be detected by sandwich ELISA with a similar sensitivity profile to WB has already been done[18].

***Refinement of analytes/reagents***

Associated with the change from WB to ELISA is the likely need to refine the analytical environment. Professor Coverley has demonstrated already that, depending on the detergents and reagents used in the process of sample preparation, in extreme conditions, the epitope (Ciz1b) can be lost. While proof-of-concept has been established, the buffer environment will need to be optimised for the ELISA test to suit the new reagent set.

***Validatory trial***

When the mAbs are available and the reagents/analytes optimised, a confirmatory trial would need to be run to validate the test in order to get CE marking. Initially, this would be a retrospective study using samples with known clinical outcomes to obtain the test sensitivity and specificity claims that would be used in marketing literature. A trial similar to that reported by Higgins et al in 2012[18] is envisaged.

**Table 5: Future development objectives**

| |
|---|
| To develop a test configuration with renewable reagents and validate on retrospective clinical sample sets |
| Prepare for prospective clinical trials to validate as confirmative test for patients with one or more indeterminate lung nodules detected by CT scan |
| Obtain CE marking |
| Obtain FDA approval (510k) |
| Initiate clinical trial |
| Prepare for marketing as a screening diagnostic test for patients at high risk of lung cancer (primarily smokers and COPD patients) |
| Develop further applications in the management of lung cancer, for example as a surrogate marker for drug efficacy and in surveillance of recurrence |
| Investigate and develop biomarker Ciz1 variant tests for other types of cancers |

*Source: Hardman & Co Life Sciences Research*

## 8. Intellectual property

Cizzle Biotechnology is protecting its technology IP through a series of four patent families surrounding the Ciz1 protein and the variant Ciz1b biomarker. Part of this series of patents has been granted in Europe and other major territories, and partially in the US. A review of the patent families was performed in 2016-17, which led to strategic and cost-cutting decisions, with one of the patent families being abandoned. The current IP is set out in Table 6.

**Table 6: Intellectual property**

| *Patent family* | *Priority date* | *Title* | *Number* | *Territory* | *Status* | *Comment* |
|---|---|---|---|---|---|---|
| **Family 1** | 05/12/2002 | Ciz1 | 2003290240 | Australia | Granted | Cover was previously |
| WO2004051269/ | | Replication | 2,507,403 | Canada | Granted | wider (with additional |
| PO43162WO | | Protein | 2316966 | Switzerland | Granted | territories). |
| | | | 2316966 | Germany | Granted | Minimal cost was |
| | | | 2316966 | Spain | Granted | maintained after |
| | | | 2316966 | France | Granted | 2016/2017 |
| | | | 2316966 | UK | Granted | Expiry date: |
| | | | 2316966 | Italy | Granted | 05/12/2023 |
| | | | 7,833,702 | USA | Granted | Future strategy |
| | | | | | is expected to be based on Families 3 and 4 | |
| **Family 2** | 05/02/2009 | Cancer | 10706706.8 | EPO | Abandoned | Abandoned |
| WO02010089559/ | | diagnosis | 61/307,479 | USA | Abandoned | when cost-cutting |
| PO43205WO | | and | 61/372,981 | USA | Abandoned | in 2016/2017 |
| | | treatment | 61/442,823 | USA | Abandoned | |
| **Family 3** | 04/08/2010 | Methods and | PCT/GB2011/001173 | | | Active |
| WO02012017208 | | compounds | | | Expiry date: | |
| | | for diagnosis | ZL201180048228.2 | China | Granted | 04/08/2031 |
| | | and | | | | |
| | | treatment | 2013-522291 | Japan | Granted | |
| | | of cancer | | | | |
| **Family 4** | 19/10/2015 | Use of a | PCT/GB2016/053203 | Australia | Filed Jun 2018 | Pending |
| P105215GB | | Fibrinogen | 2016342546 | Canada | Entry Oct 2018 | |
| | | Capture Agent | 3,002,320 | China | Filed Jul 2018 | |
| | | to detect | 201680072824.7 | EU | Published Aug 2018 | |
| | | a Ciz1b | 16784956.1 | Japan | Applied Sep 2018 | |
| | | variant | 2018-538961 | US | Filed May 2018 | |
| | | | 15/768,946 | | | |

*Source: D Young & Co LLP, Hardman & Co Life Sciences Research*

Cizzle Biotechnology uses international patent attorney, D Young & Co (Young), to represent them for all its patent requirements and applications. At the time of writing, Cizzle Biotechnology confirmed that Young had not received any correspondence relating to any potential disputes in relation to the patents/applications listed in Table 6.

The Life Sciences team at Hardman & Co has only limited experience with regard to patents. The number and geographical spread of the patents listed in Table 6, supported by the confirmation from Cizzle Biotechnology, suggests that Cizzle Biotechnology currently has a reasonable level of protection in key territories. Moreover, in the process of developing the commercial test, we would anticipate that further opportunities to reinforce its patent position are likely to arise. In addition, further comfort can be derived from the "know-how" that Cizzle Biotechnology has with regard to Ciz1 and variant Ciz1b, a specific field in which, we believe, there are only a few laboratories around the world that are currently undertaking research.

## 9. Market opportunity

Whether through CXR or chest CT scan, one of the first observations to arouse clinical suspicion is the presence of nodules in the lung on the scan. However, this simple observation does not relate directly to the presence of lung cancer. Both the size of the nodule and its rate of growth are important. Small nodules (<10mm) will probably be ignored. In order to assess the growth rate, the clinician will search medical records to see if the patient has had a previous scan. If it is the same size as that seen in previous scans, the nodule is probably unimportant or benign and will be ignored.

Guidelines[21] from the British Thorax Society offer good advice regarding nodules and provide a clear pathway for patients with nodules. Apart from the clear advice, this report has also assessed the prevalence of nodules on CXR and chest CT scans across different geographical locations, and how many of these cases

[21] Callister et al., 2015

result in a positive diagnosis of lung cancer. A summary of these data is shown in the following table. Lung nodules were found in an average of 24 per cent. of patients across the world, and 5.7 per cent. of these patients were identified as having lung cancer (1.4 per cent. of the population studied).

**Table 7: Prevalence of lung nodules and cancer by geographical location**

| *Territory* | *Studies (n)* | *Patients (n)* | *Nodule prevalence* | | *Lung cancer prevalence* | |
|---|---|---|---|---|---|---|
| | | | *Patients* | *%* | *Patients* | *%* |
| North America | 16 | 83,825 | 19,280 | 23 | 1,430 | 1.7 |
| Europe | 13 | 29,696 | 8,610 | 29 | 360 | 1.2 |
| East Asia | 2 | 24,362 | 5,100 | 36 | 80 | 0.5 |
| **Totals** | 31 | 137,883 | 32,990 | 24 | 1,870 | 1.4 |

*Source: Adapted from Callister et al[20] Hardman & Co Life Sciences Research*

***Prevalence of lung cancer in the UK***

As highlighted earlier, the number of chest CT scans performed in the year to March 2019 was ca.566,000. While not all of these will have been ordered due to the presence of previously-detected lung nodules (>3cm), many will have been. The large number of false positives will require up to four follow-up scans over the next two years, draining stretched healthcare resources. Consequently, the aim of a confirmatory *in vitro* diagnostic is to provide an accurate alternative to scanning that significantly reduces, and eventually eliminates, the number of false positives.

The following table sets out the sales potential for a reliable diagnostic biomarker test and also highlights the economic benefit to the healthcare provider through decreased follow-up chest CT scans. On the assumption that the Cizzle Biotechnology test would cost the NHS £200 (for comparison, a PSA tests costs £100, and a breast genetic/biomarker test costs £600, and a chest CT scan costs £400), the UK market potential is £20.7 million p.a. Removing 50 per cent. of the false positives from two-year follow-up would result in 207,400 fewer chest CT scans being performed, saving the NHS £83.0m, generating net savings of £62.3 million over a two-year period for the NHS.

**Table 8: UK market potential for variant Ciz1b diagnostic**

| | |
|---|---|
| Number of chest CT scans p.a. | 566,000 |
| Those associated with large nodules/high clinical suspicion | 24% |
| Potential lung cancer cases | 135,800 |
| Actual lung cancer diagnoses p.a. | 47,200 |
| No intervention | 32% |
| Remaining lung cancer patients | 32,100 |
| Potential number of false positives | 103,700 |
| Estimated cost of test | £200 |
| **UK market potential** | £20.7m |
| Reduction in those receiving follow-up by 50% | 51,850 |
| Potential reduction in chest CT scans over two-year follow-up | –207,400 |
| Cost of chest CT scan | £400 |
| Potential savings to NHS (over two years) | £83.0m |
| **Net potential saving to NHS** | £62.3m |

*Source: NHS England, BTS guidelines, Cancer Research UK, Hardman & Co Life Sciences Research*

***Prevalence of lung cancer in the US***

The US National Cancer Institute[6] (NCI) estimates that there were 1.6m patients identified with lung nodules in the US in 2019, and while many of these will have turned out to be benign or have nothing to do with cancer, 14.3 per cent., or 228,150 new cases of lung cancer will be found. Applying the same calculations as those shown in the table above to the US population, and using a test cost of $400, the sales potential of the Cizzle Biotechnology biomarker would be ca.$115m and generate potential savings for healthcare providers of ca.$230m over a two-year period.

## 10. Competitive landscape

### *Competing technologies*

A number of different technologies are trying to address the cancer diagnostics and monitoring markets. In the same way that Cizzle Biotechnology is uniquely positioned with its variant Ciz1b biomarker for the *in vitro* liquid biopsy market, other companies are uniquely positioned with their technologies (e.g. Oncimmune (ONC.L) with its autoantibody technology). Also, there are several players looking at circulating DNA from tumour cells (e.g. Angle (AGL.L)), and tests based on single nucleotide polymorphisms (SNPs) and gene panels.

**Table 9: Potential liquid biopsy competitors to Cizzle Biotechnology**

| *Autoantibody* | *Biomarkers* | *Circulating tumour cells* | *Protein biomarker* | *Genome-wide sequence variation* | *SNPs, Gene panels, Epigenetics* |
|---|---|---|---|---|---|
| Not molecular diagnostic | Not molecular diagnostic | | Conventional approach | Ultra-deep sequencing | |
| Oncimmune | Chronix Biomedical | Adaptive Biotech* | OPKO Health | Grail/Illumina+ | Epigenomics |
| | Cizzle Biotechnology | Agena Biosciences* | | | Exosome Diagnostics* |
| | Epigenomics | Angle | | | Foundation Medicine (Roche) |
| | | Biocept | | | Inivata* |
| | | Cynvenio* | | | Oxford Biodynamics |
| | | EKF Diagnostics | | | Personal Genome |
| | | Epic | | | Sysmex Inostics |
| | | Vortex Sciences* | | | |

*SNPs = Single nucleotide polymorphisms*
**Private company*
*+Illumina agreed to acquire Grail in September 2020*
*This table should not be considered comprehensive*
*Source: Hardman & Co Life Sciences Research*

The large equipment and service providers, such as Illumina, LabCorp, Roche and Quest, have not been included in the table, as their activities in liquid biopsies and/or specialist tests are very small within their groups' diverse operations. Where these companies come into play is in M&A. Smaller companies tend to take most of the risk in developing novel tests, but are approached once the technology is substantially de-risked and there is evidence of commercial success. These large players have the financial muscle and operational resources to commercialise the tests on a worldwide basis.

### *Lung cancer tests*

Many blood tests to detect tumour markers are available or under development, but many are hampered by the fact that tumour markers may also be produced by normal cells in the body. In contrast, the Cizzle Biotechnology test is based on tumour-specific technology. A number of the tests specific to lung cancer look at particular alterations of circulating DNA (cDNA) and RNA (cRNA), and are used to determine the precise type of cancer, define which therapy is more likely to work and assess the effectiveness of a particular drug. Few tests are aimed at early detection and reducing significantly the number of false positives achieved via CXT and chest CT scans.

**Table 10: Potential liquid biopsy competitors to Cizzle Biotechnology**

| *Characteristic* | *Cizzle Biotechnology* | *Epigenomics* | *Exact Sciences* | *Oncimmune* |
|---|---|---|---|---|
| Test name | Ciz1b test | Epi proLung | | Early-CDT Lung |
| Biomarker | Variant Ciz1b | SHOX2 and PTGER4 | LG3BP and C163A proteins | Circulating tumour cells |
| Technology | mAb-ELISA | Molecular diagnostic | Molecular diagnostic | ELISA |
| Identification | | | | Autoantibody |
| Regulatory position | No approval | CE marking | No approval | CE marking |

*Source: Company reports, Hardman & Co Life Sciences Research*

## 11. Overall conclusion

There is widespread recognition that the diagnosis of lung cancer needs to be made at a much earlier stage of the disease than is currently the case, which would lead to an improvement in the five-year survival rates. Even though treatment pathway guidelines are in place in many countries, they are complex and hampered by the fact that initial suspicious results from a CXR or chest CT scan, usually evidenced by the presence of lung nodules, are generally followed up with another scan and the associated high incidence of false positive results. Therefore, availability of an accurate *in vitro* diagnostic test capable of reducing the number of false positives, and the associated burden on healthcare systems, would be of enormous benefit. The advantage of the Cizzle Biotechnology technology is that the test is tumour-specific, resulting in high sensitivity and specificity. Already proven with a prototype version, the test now needs to be moved onto a high-throughput platform for commercialisation.

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## 13. Glossary

| | |
|---|---|
| AUC | Area under curve |
| Ciz1 | Cdkn1A-interacting zinc finger protein 1 |
| COPD | Chronic obstructive pulmonary disease |
| CT scan | Computed tomography (CT), sometimes called CAT scan, uses special x-ray equipment to obtain image data from different angles around the body, and then uses computer processing of the information to show a cross-section of body tissues and organs. |
| CXR | Chest X-ray |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| IP | Intellectual property |
| NSCLC | Non-small cell lung cancer |
| ROC | Receiver operating characteristic. ROC curves are used to see how any predictive model can distinguish between the true positives and negatives. In order to do this, a model needs to not only correctly predict a positive as a positive, but also a negative as a negative. ROC curves achieve this by plotting sensitivity (probability of predicting a real positive will be a positive) against specificity (probability of predicting a real negative will be a positive). |
| SCLC | Small cell lung cancer |
| SNP | Single nucleotide polymorphisms |
| USPSTF | US Preventive Services Task Force |
| WB | Western blot |