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Evaluation Report on Roquefort Therapeutics plc

Realising Midkine's potential

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Roquefort Therapeutics plc is listed on the London Stock Exchange (LSE:ROQ). LYRAMID is a drug developer focused on the therapeutic potential of a protein called Midkine, which may be useful as a target in the treatment of severe inflammatory and autoimmune disorders, and cancer, among other disease conditions. An ASX-listed biotech called Cellmid spent many years developing Midkine-based drugs. In April 2021 these programmes were spun out as an independent company called LYRAMID. In December 2022 Roquefort Therapeutics acquired the entire issued capital of LYRAMID and raised £3m to fund pre-clinical drug development for the Midkine programme.

Pursuing multi-billion dollar market opportunity

LYRAMID sees immediate potential for Midkine in two key areas: overcoming resistance to cancer immunotherapy and treatment of COVID-19. Both are multi-billion dollar market opportunities. The COVID-19 opportunity is significant given the future unmet medical need for preventing infected patients progressing to acute respiratory and multi-organ failure, as well as the debilitating symptoms of Long COVID.

A cancer immunotherapy breakthrough

The immune checkpoint inhibitor drugs have been game-changing. However, they don't work for every patient. LYRAMID is pursuing early-stage science which suggests that Midkine inhibition can overcome treatment resistance for these cancer patients. The upside is in the billions.

Plenty of upside beyond COVID-19 and cancer

Once new lead Midkine drugs have been identified, LYRAMID can go after its initial programmes in COVID-19 and cancer, and follow that with programmes in autoimmunity and inflammatory conditions such as Chronic Kidney Disease. A selection of nine publicly traded companies that are roughly comparable to LYRAMID suggests a valuation of up to ~US$135m (optimistic case). Key risks we see in LYRAMID include: 1) clinical risk; 2) timing risk; and 3) uptake risk.

"We think Roquefort Therapeutics can take Midkine as a drug target to the next stage, and ultimately bring new drugs to market"

- Associate Professor Graham Robertson, CSO of LYRAMID

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Disclosure: Pitt Street Research directors own shares in Roquefort Therapeutics plc.

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Introducing Roquefort Therapeutics

LYRAMID, a wholly owned subsidiary of Roquefort Therapeutics, is a drug developer focused on the therapeutic potential of a protein called Midkine. A large body of work over the last two decades has established that Midkine, variously described as a growth factor or as a cytokine, is potentially useful as a target in the treatment of severe inflammatory and autoimmune disorders, and cancer, among other disease conditions. An ASX-listed biotech called Cellmid[1] spent many years developing Midkine-based drugs covered by an extensive patent portfolio around Midkine. In April 2021 these programmes were spun out as an independent company called LYRAMID[2]. In December 2022 Roquefort Therapeutics acquired the entire issued capital of LYRAMID and raised £3m to fund pre-clinical drug development for the Midkine programme.

Midkine is a player in multiple disease conditions including cancer

What is Midkine? Midkine, a heparin-binding protein[3], has long been known to be important in embryonic development. While barely detectable in healthy adults, Midkine is highly expressed in cancer, inflammatory conditions and autoimmune disorders. In addition to Midkine's role in preventing tumour cell death, it promotes metastatic spread to other organs, tumour angiogenesis, cell growth, and resistance to chemotherapy, thereby contributing to various levels of cancer progression and reduced patient survival. Importantly, Midkine hinders the normal immune response to tumours, rendering the blockbuster cancer immunotherapy drugs ineffective. Overcoming resistance to immune checkpoint inhibitors is one of the current 'holy grails' of cancer treatment. In the context of chronic inflammatory and autoimmune diseases, Midkine is switched on at the onset of inflammatory responses, making it an ideal 'upstream' target in diseases such as inflammatory kidney and heart disease, as well as the autoimmune disorders Rheumatoid Arthritis, Crohn's disease, lupus and Multiple Sclerosis. Midkine also contributes to various lung diseases and may be a key driver of lung and multi-organ pathologies involved in COVID-19.

Roquefort Therapeutics will be the dominant player in the Midkine space. Midkine was discovered in 1988 at Nagoya University in Japan by Professors Muramatsu and Kadomatsu[4]. The intellectual property developed at Nagoya was acquired by a Japanese private company called Cell Signals in 2001. Cell Signals, funded by venture capital, further developed the Midkine assets and eventually sold these to Cellmid in 2008[5]. That company continued the research and development of Midkine therapeutics, and it developed, validated and CE marked a Midkine diagnostic assay for the accurate measurement of Midkine in blood. The Cell Signals acquisition came with a library of around 120 proprietary anti-Midkine antibodies and a comprehensive patent portfolio protecting Midkine and Midkine antagonists globally. LYRAMID inherited this Midkine venture in 2021. We look at the 2008-2021 history of the Midkine programmes in Appendix V of this note.

In 2022 Roquefort Therapeutics will work on building its pipeline of Midkine-based drugs. The company sees immediate potential for Midkine in two key areas: overcoming resistance to cancer immunotherapy, and treatment of COVID-19. LYRAMID's new management, led by CSO Assoc.

[1] ASX: CDY, This Sydney-based company changed its name to Anagenics, ASX: AN1, in late 2021 – see anagenics.com
[2] Allowing it to focus on its portfolio of anti-aging and hair care products.
[3] Midkine is 13 kilodaltons or 121 amino acids in size. There are two Midkine domain, called 'C' and 'N'. Midkine has >90% amino acid identity between mammalian species.
[4] Biochem Biophys Res Commun. 1988 Mar 30;151(3)1312-8.
[5] For US$1.5m and 20 million shares.

Professor Graham Robertson, has also come to the view that gene silencing technologies represent a better way to block Midkine than antibodies, and is working with key international experts with expertise in developing nucleic acid-based drugs.

How will Rocquefort Therapeutics realise shareholder value with its Midkine programmes? We see Roquefort Therapeutics creating value from its portfolio in the near term by gaining *in vivo* experimental proof of concept and then moving lead drug candidates into pre-clinical development. There is potential in the near term for early partnering deals for new therapeutic products covered by novel composition of matter patents, and method patents.

Seven reasons to look at Roquefort Therapeutics

1. **LYRAMID dominates an important drug development target.** A large body of work has established Midkine as a player in various disease conditions, including severe inflammatory and autoimmune disorders, and cancer. LYRAMID represents the company with the most investment in this target.
2. **LYRAMID has inherited in excess of 12 years work on Midkine** that was invested by the vendor between 2008 and 2020. That work established valuable intellectual property, provided validation of the importance of Midkine as a target, and has guided the current drug development priorities.
3. **LYRAMID may have the basis of a new drug for COVID-19.** The evidence from various sources of Midkine's role in the organ damage and inflammation associated with COVID-19 infection suggests the potential for the company to develop a drug to treat infections.
4. **LYRAMID can potentially help overcome treatment resistance in cancer immunotherapy.** Evidence is beginning to emerge that Midkine creates a tumour microenvironment that enables cancers to evade the immune system. LYRAMID believes that a Midkine inhibitor can therefore help blockbuster drugs such as Yervoy, Keytruda and Opdivo to work in more patients. Such a drug could itself reasonably become a blockbuster.

LYRAMID can build a significant pipeline of drug candidates

5. **LYRAMID can build a significant pipeline of drug candidates**, given the utility of Midkine inhibition across multiple indications in the cancer, autoimmune and kidney disease space.
6. **LYRAMID is perfecting a better targeting approach for Midkine**, working with experts in gene silencing drugs in the conviction that such drugs can be superior to the monoclonal antibody drugs which the vendor developed. LYRAMID's new drugs will allow novel composition of matter and method patents.
7. **The news flow is likely to be solid for LYRAMID**, with progress expected on development of new drug candidates, on the lead programmes in COVID-19 and cancer treatment resistance, and on the publication of research work related to Midkine.

LYRAMID's current focus with Midkine in COVID-19, in cancer and in other indications

An initial focus of LYRAMID will be on COVID-19

After LYRAMID was established in 2021, the new management under Associate Professor Graham Robertson, Chief Scientific Officer, focused the Midkine programme on COVID-19, cancer, and chronic inflammatory conditions such as chronic kidney disease (CKD) and autoimmunity. The company also made the strategic decision to investigate alternative drug modalities for Midkine inhibition, moving away from monoclonal antibodies as its preferred approach in drug development. While antibodies are important tools for validation of a disease target, such as Midkine, they are expensive and can be high risk when it comes to a commercial drug development programme.

COVID-19. The discovery that SARS-CoV-2, the virus that causes the COVID-19 disease, infects lung cells via the ACE2 receptor suggested that Midkine inhibitors could potentially reduce the consequences of viral infection starting in lung cells. LYRAMID is pursuing two angles here. Firstly, that a Midkine inhibitor could rebalance the Renin-Angiotensin System associated with ACE2, and thereby reduce downstream lung and other organ failure. Secondly, that Midkine could prevent 'NETosis', a deleterious inflammatory process involving neutrophils that has been found to occur in the lungs of severe COVID-19 patients. NETosis has recently been shown to contribute to excessive clotting of lung capillaries, leading to respiratory failure in COVID-19 due to blocked blood vessels[6].

Cancer immunotherapy resistance. A key 2020 paper from the laboratory of Professor Marisol Soengas at CNIO, the Spanish National Cancer Research Centre in Madrid[7], has shown that Midkine is involved in blunting the normal anti-tumour immune response in melanoma. This highlights the potential for Midkine to sensitise melanoma to respond to immune checkpoint inhibitor therapy[8]. Overcoming immunotherapy resistance will be of immense benefit for the 50% of melanoma patients, and up to 90% of patients with other tumour types whose tumours are resistant to the immune checkpoint inhibitors Yervoy, Keytruda and Opdivo.

Other indications in inflammation and autoimmune disease. LYRAMID will assess the feasibility of a programme focusing on inflammation associated with chronic heart failure. Findings published in 2019 demonstrated that blocking Midkine prevented heart muscle damage from inflammation in myocarditis. Dr Ludwig Weckbach a research scientist/cardiologist at Ludwig Maximilian University of Munich in Germany, established the link between Midkine and NETosis[9] by showing the efficacy of Midkine antibodies inhibit NETosis in mice with heart failure due to myocarditis.

Chronic Kidney Disease (CKD), potentially. Many studies have shown that Midkine contributes to several forms of CKD by promoting renal inflammation. Blocking Midkine with various reagents including LYRAMID's therapeutic antibodies, has consistently been beneficial in reducing kidney injury, inflammation and fibrosis while restoring renal function. CKD is a

[6] See Blood. 2020 Sep 3; 136(10): 1169–1179.
[7] CNIO stands for Centro Nacional de Investigaciones Oncológicas. For the Soengas lab see cnio.es/en/personas/maria-s-soengas-2.
[8] Cerezo-Wallis et. al. (2020), *Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state*. Nat Med. 2020 Dec;26(12):1865-1877. Epub 2020 Oct 19.
[9] Weckbach et. al. (2019), *Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis*. J Exp Med. 2019 Feb 4;216(2):350-368. Epub 2019 Jan 15.

clinical area of major unmet need, with multiple reports indicating that it is becoming a very significant global health problem.

LYRAMID believes directly blocking Midkine expression represent a better approach to Midkine antagonism

LYRAMID is investigating the best approach to Midkine antagonism. For many years LYRAMID's predecessors worked on monoclonal antibodies that targeted Midkine. Since the spin-out, LYRAMID has progressed work on gene silencing therapeutics as an alternative drug development strategy. Drugs that directly target Midkine expression are likely to work more effectively than antibodies as their mode of action is not dependant on the precise molecular shape of the Midkine protein[17] within diseased tissues. There is already considerable experimental evidence that reagents that block Midkine expression are highly effective in cancer and kidney disease i*n vivo*[10]. It is this data that LYRAMID intends to build on with its current programme.

LYRAMID Programme No. 1: A potential treatment for COVID-19

Midkine inhibition can potentially treat COVID-19, by acting on the Renin-Angiotensin System, as well as blunting 'NETosis'. In addition to Acute Respiratory Distress Syndrome (ARDS)[11], COVID-19 causes multi-organ failure through three distinct disease processes involving: dysregulation of the Renin Angiotensin System; excessive blood clotting within the tissues of vital organs; and the cytokine storm associated with SARS-CoV-2 infection. Looking at the detailed studies into the pathology of COVID-19 as it emerged through 2020 and 2021, LYRAMID believes that Midkine inhibition could represent a valuable anti-COVID-19 drug because of multiple mechanisms of action. Inhibiting Midkine may:

i) downregulate ACE and thereby restore balance to the Renin-Angiotensin System;

ii) reduce the excessive inflammation associated with the cytokine storm; and

iii) prevent neutrophil recruitment and activation (ie NETosis) that underpins damaging blood clots in lungs and other organs.

The Renin-Angiotensin System isn't just important for hypertension and kidney disease

What is the Renin-Angiotensin System (RAS)? Standard management of hypertension involves the Renin-Angiotensin System (RAS), since the so-called ACE inhibitor drugs such as Captopril work through modulating this system. Renin is a hormone produced by the kidneys whenever the body senses that blood pressure has become too low. Renin then converts angiotensinogen into Angiotensin I, which is in turn converted by Angiotensin-Converting Enzyme (ACE) into Angiotensin II. This active hormone is a powerful vasoconstrictor, that is, it constricts blood vessels, which in turn increases blood pressure. The ACE inhibitors act by blocking production of Angiotensin II so that this vasoconstriction is reduced, thereby reducing blood pressure. However, the Renin-Angiotensin System isn't just important for hypertension and kidney disease. In recent years a large body of knowledge has been built up showing that activation of Angiotensin I and Angiotensin II causes a number of other unfavourable effects ultimately leading to organ damage[12].

[10] Cancer Res. 2001 Dec 1;61(23):8486-91.
[11] The rapid build-up of fluid in the air sacs in the lungs, preventing oxygen to reach the bloodstream.
[12] See Iwanami et. al (2009), *Inhibition of the Renin-Angiotensin System and target organ protection*. Hypertens Res. 2009 Apr;32(4):229-37. Epub 2009 Feb 27.

How is COVID-19 related to the Renin-Angiotensin System? The surface of the SARS-CoV-2 virus is studded with a glycoprotein[13] called 'Spike'. Very shortly after the COVID-19 Pandemic began, it was established that the Spike protein allows the SARS-CoV-2 virus to gain entry into lung cells via the ACE2 receptor that sits on the surface of those cells. ACE2 is abundantly expressed in a variety of cells residing in many different human organs including the lungs. The link between COVID-19 and ACE2 also helps provide an explanation for the heart failure in the acute stage that appears in severe cases of COVID progression[14]. The virus enters the cell and depletes existing ACE2. The lack of ACE2 then leads to an imbalanced Renin-Angiotensin System. This in turn progresses to damage in other organs besides the lungs such as the heart and kidney[15].

Midkine upregulates ACE2, which COVID-19 uses to enter cells

How is Midkine involved in the Renin-Angiotensin System? A seminal study in 2009 showed that Midkine upregulates ACE expression within the lung[16] in the presence of renal failure, and that Midkine inhibition can reduce the hypertension associated with increased ACE[17].

What is NETosis and how is it relevant in COVID-19? NETosis is the process whereby the immune system kills invading pathogens using structures called 'Neutrophil Extracellular Traps' or 'NETs'. Neutrophils are white blood cells from the innate immune system, which is the body's first line of defence against microbial pathogens. Originally neutrophils were understood to work via phagocytosis, where the neutrophils eat the pathogens. In 2004 the Neutrophil Extracellular Trap was established as a newly understood mechanism whereby neutrophils kill microbes by extruding material poisonous to the pathogen[18]. NETosis is the process of NET formation. Like most aspects of the immune system, neutrophils with their NETS can be a two-edged sword. They can kill pathogens, but they can also lead to a deleterious vicious cycle of chronic inflammation that may result in organ failure. This has become apparent in COVID-19 where not only organ failure, but blood clots, have been associated with NETosis[19]. Clinical studies have revealed that 'Long COVID', where patients recover from the acute infection but experience long term symptoms, may also be attributed to NETosis[20].

The next steps for LYRAMID in its COVID-19 programme. LYRAMID is currently developing a novel and effective way to block production of functional Midkine by the body. Once developed, these novel drug candidates will be tested in an *in vitro* environment for their efficacy in inhibiting SARS-CoV-2 infection as well as *in vivo* pre-clinical models of COVID-19 disease progression. Success in this setting would then move the lead candidates into clinical development. While severe COVID-19 rates are likely to decline over the next two years as initial vaccine rollout is followed by booster shots, new variants of SARS-CoV-2 virus will inevitably continue to emerge for many years, leading to ongoing patient cohorts requiring new treatments.

[13] As the word suggests, a sugar-protein combination.
[14] See Beyerstedt et. al. (2021), *COVID-19: Angiotensin-Converting Enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection*. Eur J Clin Microbiol Infect Dis. 2021 Jan 3 : 1–15. [Epub ahead of print]
[15] See Vaduganathan et. al. (2020), *Renin-Angiotensin-Aldosterone System Inhibitors in Patients with COVID-19.* N Engl J Med. 2020 Apr 23;382(17):1653-1659. Epub 2020 Mar 30.
[16] See Kadamatsu (2010), *Midkine regulation of the renin-angiotensin system.* Curr Hypertens Rep . 2010 Apr;12(2):74-9.
[17] Hobo et. al. (2009), *The growth factor Midkine regulates the renin-angiotensin system in mice*. J Clin Invest. 2009 Jun;119(6):1616-25. Epub 2009 May 18.
[18] See Brinkmann et. al. (2004*), Neutrophil extracellular traps kill bacteria*. Science. 2004 Mar 5;303(5663):1532-5.
[19] See Gillot etl. Al. (2021), *NETosis and the Immune System in COVID-19: Mechanisms and Potential Treatments* Front. Pharmacol. 2021 Aug 5;12:708302.
[20] See Sawadogo et. al. (2020), *How NETosis could drive "Post-COVID-19 syndrome" among survivors*. Immunol Lett. 2020 Dec;228:35-37. Epub 2020 Sep 29.

LYRAMID Programme No. 2: Overcoming treatment resistance in cancer immunotherapy by targeting Midkine.

Midkine inhibitors may be able to improve cancer immunotherapy

Midkine inhibitors may improve cancer immunotherapy. The most significant paradigm shift in the treatment of cancer has been the recent emergence of cancer immunotherapies, where the patient's own immune system is harnessed to attack the tumour cells. LYRAMID is currently following through ground-breaking studies showing that Midkine inhibition may improve cancer immunotherapy treatment outcomes by overcoming immune checkpoint inhibitor resistance.

The immune checkpoint inhibitors have revolutionised cancer treatment. The cancer immunotherapy revolution effectively started in March 2011 when Bristol-Myers Squibb gained FDA approval for Yervoy[21], for the treatment of advanced melanoma. Yervoy was the first of the so-called 'immune checkpoint inhibitor' drugs which work by taking the brakes off anti-tumour immune responses. This drug's success – in Phase 3 it increased survival in advanced melanoma by >50%[22] – prompted other programs to follow with different immune checkpoint inhibitors. There are now seven that have gained FDA approval – as well as Yervoy we now have Merck and Co's Keytruda[23], BMS's Opdivo[24], Roche's Tecentriq[25], Merck KGaA and Pfizer's Bavencio[26], AstraZeneca's Imfinzi[27] and Regeneron and Sanofi's Libtayo[28]. They are now used to treat many cancers and a number are either blockbusters or headed that way. Keytruda enjoyed US$14.4bn in sales in 2020 while Opdivo did US$7.0bn.

Cancer immunotherapy treatments don't work for everybody, but the search is on to increase their effectiveness. The immune checkpoint inhibitors (ICIs) have been game-changing anticancer drugs. However, they don't work for every patient with many only gaining minimal or no benefit in terms of recurrence and long-term survival. Around 50% of melanoma patients do not respond to ICIs, while up to 90% of patients with other tumours do not gain any benefit. Typically for melanoma, at the five year mark only around a fifth or a quarter of patients are still alive[29]. Investigators and pharma companies are now vigorously pursuing drugs that would work in combination with the immune checkpoint drugs to overcome resistance and improve survival rates. LYRAMID believes there is strong evidence that Midkine inhibition represents a viable approach to the immunotherapy resistance problem. Cancer immunotherapy may ultimately become a US$100bn market in the middle of the current decade[30], so any success LYRAMID can enjoy opens up a truly massive opportunity.

A key 2020 paper showed that Midkine could 'rewire' macrophages to become tolerant to cancer

There has been evidence for some years for Midkine's role in melanoma. Melanoma was where the early checkpoint inhibitor drugs were first investigated because skin cancer has traditionally been the 'model' tumour for understanding tumour immune responses, and melanoma was long known to be amenable to immunotherapy[31]. In 2017 Marisol Soengas at the

[21] Generic name ipilimumab, see yervoy.com.
[22] See N Engl J Med. 2010 Aug 19;363(8):711-23. Epub 2010 Jun 5.
[23] Generic name pembrolizumab, first FDA approved in September 2014. See keytruda.com.
[24] Generic name nivolumab, first FDA approved in December 2014. See opdivo.com.
[25] Generic name atezolizumab, first FDA approved in May 2016. See tecentriq.com.
[26] Generic name avelumab, first FDA approved in March 2017. See bavencio.com.
[27] Generic name durvalumab, first FDA approved in May 2017. See imfinzi.com.
[28] Generic name cemiplimab, first FDA approved in September 2018. See libtayo.com.
[29] See, for example, J Clin Oncol. 2019 Oct 1;37(28):2518-2527. Epub 2019 Jun 2.
[30] See the Scancell presentation entitled *At the Forefront of Immuno-Oncology*, dated 17July 2019, at scancell.co.uk.
[31] See Ann Oncol. 2012 Sep;23 Suppl 8:viii10-4.

CNIO was looking into the mechanisms by which melanoma can metastasise through the body via the lymphatic system. What the Soengas lab found was that Midkine, which has long been known to be elevated in cancer, induced the formation of new lymphatic vessels that, in turn, act as the conduit to spread the tumour to other parts of the body. This work was important enough to warrant publication in the prestigious journal *Nature*[32]. Further work by the Spanish group led to the pivotal discovery in 2020 that Midkine secreted by melanomas can help create a tumour immune microenvironment that enables the melanoma to evade the immune system. Specifically, Midkine 'rewired' macrophages to become tolerant to the cancer so that the immune system could not generate the $CD8^+$ T cells that would normally kill cancer cells. That changed, however, when Midkine inhibitors were used, permitting the immune checkpoint inhibitors to work as expected. This seminal work, demonstrating that pharmacological blockade of Midkine overcame immunotherapy resistance, was published in *Nature Medicine*[33].

We look to see more evidence of overcoming treatment resistance in other cancers. If Midkine antagonism works to overcome immunotherapy resistance in melanoma it can potentially be of benefit for other tumour types. Recent studies found that a signature of elevated Midkine associated with immunotherapy resistance is a feature of gliomas, kidney and lung cancer[40]. We expect LYRAMID and its academic collaborators can build out this evidence as a way of establishing the value of anticancer drugs based on Midkine inhibition in this space.

The path forward for LYRAMID is relatively straightforward. The immune checkpoint inhibitors have now built up many years of clinical use in multiple combinations. What LYRAMID proposes is development of an appropriate Midkine drug, which can then be moved into pre-clinical testing alongside an approved immune checkpoint inhibitor. Once *in vivo* efficacy is established in appropriate animal tumour models, clinical trials can be conducted in combination treatment regimens with those same checkpoint inhibitors.

Midkine as a target in cancer

Midkine promotes tumour cell proliferation and migration in cancer

Midkine is an ideal cancer target. A protein is a particularly good cancer target if it is a) expressed in many different tumour types, b) not present in healthy people, and c) if it can be found to play a role in cancer at various stages. Midkine is ideal in this regard because:

- Midkine is highly expressed in around 30 malignant tumour types[34] but is almost absent in healthy people[35];
- Midkine rewires tumour immune responses leading to immunotherapy resistance;
- The protein inhibits apoptosis of cancer cells[36];
- The presence of elevated Midkine in some cancer patients is diagnostic, as well as predictive of treatment response and prognostic of poor outcome and reduced survival[37];

[32] Olmeda et. al. (2017), *Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of Midkine*. Nature. 2017 Jun 28;546(7660):676-680.
[33] Cerezo-Wallis et. al. (2020), *Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state*. Nat Med. 2020 Dec;26(12):1865-1877. Epub 2020 Oct 19.
[34] See Cellmid's 31 March 2014 presentation, slide 16.
[35] See Ikematsu et. al. (2000), *Serum Midkine levels are increased in patients with various types of carcinomas*. Br J Cancer. 2000 Sep;83(6):701-6.
See Br J Pharmacol. 2014 Jun;171(12):2925-39. Epub 2014 Jan 27. This paper is authored by Darren Jones, who was Cellmid's Head of Product Development between 2009 and 2015.
[36] See PLoS One. 2013 Aug 16;8(8):e71093.
[37] Mol Med Rep. 2012 Feb;5(2):415-9. Epub 2011 Oct 31.

- The protein promotes tumour cell proliferation and migration in cancer[38];
- Midkine drives tumour angiogenesis[39];

Midkine antagonists work in cancer. Consider just five papers:

- Takei showed in 2001 that antisense oligonucleotides to Midkine were highly effective at suppressing tumour growth in a mouse model of rectal carcinoma[40];
- Sueyoshi showed in 2011 that Midkine antibodies inhibited primary tumour growth and slowed metastasis in a xenograft model of osteosarcoma [41];
- Kishida showed in 2012 that a Midkine aptamer strongly suppressed neuroblastoma tumour growth in a mouse xenograft model[42];
- In their 2017 nature paper the Soengas group demonstrated the pivotal role for Midkine in melanoma metastasis using siRNA to silence Midkine[43];
- Cerezo-Wallis published seminal findings in *Nature Medicine* in 2020 that siRNA targeting Midkine, as well as a small molecule inhibitor, blocked Midkine from reconfiguring melanoma immune responses and restored efficacy of immune checkpoint inhibitors such as Keytruda[44].

Midkine as an inflammation target

There is a large body of work showing Midkine to be a driver of inflammation and that antagonists of Midkine have the potential to be potent anti-inflammatory drugs for many different diseases. Consider four prominent examples from the literature[45] with additional confirmation from in-house studies presented in registered patents:

- **Rheumatoid Arthritis (RA)**. In 2004 Maruyama showed, in a mouse model of RA, that Midkine knockout mice seldom developed RA, while most of the wild-type mice did[46];
- **Multiple Sclerosis**. In 2008 Wang et. al. demonstrated that anti-Midkine aptamers could alleviate Experimental Autoimmune Encephalomyelitis – the standard animal model for MS – by expanding the regulatory T cell population[47] that normally counters autoimmune responses. Previously, Celllmid had established that Midkine antibodies work in this setting to reduce inflammation and the clinical score of MS symptoms[48];
- **Kidney inflammation**. In 2005 Sato et al. showed that kidney inflammation and damage were suppressed when mice were given anti-Midkine oligonucleotides.[49]

Midkine inhibition is beneficial in myocarditis

- **Chronic inflammatory heart failure**. In 2019 LYRAMID's collaborator Ludwig Weckbach directly demonstrated the value of pharmacologically

[38] See Mol Cancer Res. 2014 May;12(5):670-80. Epub 2014 Feb 24.
[39] See Cancer Res. 1997 May 1;57(9):1814-9.
[40] Cancer Res. 2001 Dec 1;61(23):8486-91.
[41] See Cancer Lett. 2012 Mar;316(1):23-30. Epub 2011 Oct 20.
[42] See Cancer Res. 2013 Feb 15;73(4):1318-27. Epub 2012 Dec 14.
[43] Olmeda et. al. (2017), *Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of Midkine*. Nature. 2017 Jun 28;546(7660):676-680.
[44] Cerezo-Wallis et. al. (2020), *Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state*. Nat Med. 2020 Dec;26(12):1865-1877. Epub 2020 Oct 19.
[45] There are many others. Consider, for example, the work of Narita et. al., who in 2008 showed that Midkine is expressed by infiltrating macrophages in in-stent restenosis, making Midkine antibodies potentially very valuable in preventing such restenosis. See J Vasc Surg. 2008 Jun;47(6):1322-9. Epub 2008 Mar 19.
[46] See Arthritis Rheum. 2004 May;50(5):1420-9.
[47] See Proc Natl Acad Sci U S A. 2008 Mar 11;105(10):3915-20. Epub 2008 Mar 4.
[48] WO/2007/055378, priority date 14 November 2005.
[49] See Kidney Int. 2005 Apr;67(4):1330-9.

blocking Midkine using LYRAMID's Midkine antibodies to prevent inflammation and fibrosis, while preserving cardiac performance in a mouse model of autoimmune myocarditis[50].

LYRAMID may have the Next Big Thing in kidney disease

There is ample pre-clinical evidence that Midkine antagonists would work in kidney disease:

- In 2001 Sato et al. showed that Midkine facilitated neutrophil infiltration in ischemic renal injury[51];
- In 2006 and 2007 studies by Kosugi et. al. showed that Midkine is a key driver in the pathogenesis of diabetic nephropathy[52];
- In 2011 Kato et. al. showed that Midkine-driven neutrophil infiltration was involved in the protein overload model of acute kidney injury[53]

The markets for kidney disease are large, growing and underserved:

- 15% of the US adult population or 49 million people have chronic kidney disease[54], largely driven by the rapid rise of Type II diabetes prevalence;
- The incidence of End Stage Renal Disease in the US increased to 132,000 in 2018[64];
- There are no on-market specific disease modifying therapies, beyond steroids.

LYRAMID has various renal indications in mind for Midkine based drugs, including:

- **Diabetic nephropathy**, a problem for around a third of all diabetics[55] exacerbated by the ever-increasing prevalence of Type II diabetes.
- **Glomerular sclerosis**, which could allow LYRAMID to gain Orphan Drug status - in the US perhaps 70,000 people have the most common type of this condition, called Focal Segmental Glomerulosclerosis[56].

Glomerular sclerosis could allow LYRAMID to gain Orphan Drug status

Lupus nephritis may represent an indication worth pursuing in the medium term. The FDA approval in 2011 of GSK's Benlysta[57] opened up a new era for the treatment of Systemic Lupus Erythematosus (SLE), since this was the first new drug for lupus in almost sixty years. Lupus remains, however, an area of significant unmet medical need. Notably, the GSK drug can't be used for the treatment of the renal disease – the condition called lupus nephritis – or severe central nervous system involvement of SLE. In 2017 a team at Nagoya University in Japan established that Midkine was a player in lupus nephritis through promoting the relevant T cell activation as a driver of autoimmunity[58]. LYRAMID intends to explore the potential of Midkine antagonists in lupus nephritis. There may be a place for including lupus

[50] See Weckbach et. al. (2019), *Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis*. J Exp Med. 216:350-368.
[51] See J Immunol. 2001 Sep 15;167(6):3463-9.
[52] See Lab Invest. 2007 Sep;87(9):903-13. Epub 2007 Jul 2.
[53] See Clin Exp Nephrol. 2011 Jun;15(3):346-54. Epub 2011 Mar 1.
[54] Source: US Renal Data System, 2020 Annual Data Report.
[55] Consider that ~20-30% of previously diagnosed diabetics have microalbuminuria (see Am J Kidney Dis. 2002 Mar;39(3):445-59), the earliest stage of nephropathy. Around 5% will have macroalbuminuria and 0.8% elevated plasma creatinine, where the kidney is failing and the annualised risk of death is ~20% (see Kidney Int. 2003 Jan;63(1):225-32).
[56] This was the disease which stunted the growth of the American actor Gary Coleman (1968-2010), famous for playing Arnold in the 1980s situation comedy Diff'rent Strokes.
[57] Generic name belimumab, see benlysta.com.
[58] See Maruda et. al. (2017), *Growth factor Midkine promotes T cell activation through Nuclear Factor of Activated T Cells signaling and Th1 cell differentiation in Lupus Nephritis*. Am J Pathol. 2017 Apr;187(4):740-751. Epub 2017 Feb 7.
[71] Jones DR, 2014. *Measuring Midkine: the utility of Midkine as a biomarker in cancer and other diseases.* Brit J Pharmacol 171:2925

nephritis as a condition linked to autoimmune SLE, and the company has had discussions with an SLE expert around this.

Midkine makes for great diagnostics

Midkine antibodies are more specific and sensitive for cancer that other well-known biomarkers

Midkine makes for a great cancer biomarker[71]. We noted above that serum Midkine is elevated in many different types of cancer. However, what makes Midkine a great cancer biomarker is evidence that it is elevated in the very early stages of cancer, is prognostic for poor patient outcome and predictive for treatment response. Various studies have suggested that Midkine antibodies could be more specific and sensitive for biomarkers for cancer than other, better-known biomarkers.

- In 2017 Olmeda et al. showed that the levels of Midkine in lymph node metastases was prognostic for subsequent recurrence of melanoma over 10-15 years. 60% of all patients with high Midkine developed distal metastases compared to 15% of patients with low Midkine.[59];
- In 2017 Jia et al. demonstrated that serum Midkine was a prognostic marker for thyroid cancer metastases[60];
- In 2003 Shimada et. al. showed that serum Midkine could function as an effective prognostic marker for oesophageal cancer[61];
- In 2009 Ibusuki et. al. showed that the same approach worked in breast cancer[62].

LYRAMID inherits a Midkine ELISA which was launched in November 2010 for use in research applications. This ELISA has an 8 pg/mL limit of detection, well within the range of healthy serum Midkine levels that can run as high as 500 pg/mL[63]. The product gained its CE Mark in October 2011. It is validated for measuring circulating Midkine levels in patient sera and has been used extensively by LYRAMID's academic and industry collaborators in clinical studies for multiple cancers and other disease indications.

There have been three licensing deals for its Midkine diagnostic:

- A diagnostic for early detection of lung cancer was licensed to Celera in October 2009. Quest Diagnostics[64] bought Celera in 2011 for US$344m;
- A bladder cancer diagnostic was licensed to Pacific Edge[65] in May 2010 and launched in the US in March 2013;
- A Midkine early cancer diagnostic was licensed to Japan's Fujikara Kasei in July 2013[66].

LYRAMID already has a future companion diagnostic in its portfolio

The biomarker work is relevant to future LYRAMID cancer therapeutic programmes, since for optimal treatment outcomes any successful drug will, like Herceptin and other anti-cancer reagents, need a companion biomarker to identify those patients who will benefit most from the drug.

[59] Nature 2017 546:676.
[60] See Jia et al (2017) *Serum Midkine as a surrogate biomarker for metastatic prediction in differentiated thyroid cancer patients*. Scientific Reports 7:43516.
[61] See Cancer Sci. 2003 Jul;94(7):628-32.
[62] See Cancer Sci. 2009 Sep;100(9):1735-9. Epub 2009 Jun 1. The markers were CA15-3, CEA and NCCST-439.
[63] Cellmid has reported that the inter-assay CV of its ELISA is less than 25% and that the test was accurate with a recovery range of between 75% and 125%. Moreover there was no cross-reactivity to pleiotrophin or other serum components.
[64] Madison, NJ, NYSE: DGX, www.questdiagnostics.com.
[65] Dunedin, New Zealand, NZX: PEB, www.pacificedge.co.nz. This company recently cross-listed on ASX.
[66] See www.fkkasei.co.jp.

Summary

LYRAMID inherits a large body of work on Midkine, where its predecessors have been able to show since 2008 that Midkine inhibition can be important in cancer, inflammatory heart failure, autoimmunity and kidney disease.

LYRAMID is now building out its pipeline of Midkine-based drugs with lead programmes in pre-clinical development for COVID-19 and cancer immunotherapy.

LYRAMID is exploring better ways to drug Midkine, with the company working with key international experts in gene silencing technologies.

Appendix I - A LYRAMID glossary

Antibodies – Immune system proteins that can bind to antigens and help to neutralise the potentially harmful effects of the cells carrying the antigen.

Angiogenesis – The process underlying the formation of new blood vessels, including the blood vessels which feed tumours.

Antigen – The 'bad guy' substance that stimulates the immune system to respond to the perceived threat. An antigen is the protein to which antibodies bind.

Apoptosis – 'Programmed' cell death, that is, cell death that is naturally-occurring. Cancer cells tend to avoid apoptosis.

Aptamer – A nucleic acid molecule that binds to a specific target molecule.

Atherosclerosis – The clogging or hardening of blood vessels caused by plaques of fatty deposits, usually cholesterol.

Autoimmune disease – Disease in which the immune system is attacking 'self' antigens rather than 'non-self antigens'.

Biomarker – A natural substance used as an indicator of a biological state, especially to detect the presence or severity of disease. There are three types of biomarker: diagnostic for detection of a specific disease; prognostic for indicating future development of the disease; and predictive for predicting the response to treatment.

Composition of matter – A patent that covers the chemical make-up of a drug.

Cytokines – Proteins secreted by white blood cells involved in activating various other immune system cells. Cytokines are often called an 'immunomodulating proteins' due to their role in immune system regulation.

Diabetic nephropathy – Kidney damage resulting from diabetes, which often progresses to kidney failure.

ELISA – Short for Enzyme-Linked ImmunoSorbent Assay, a test method for detecting antigens in blood or other biological fluids that involves the recognition of the antigen by specific antibodies. ELISAs represent a way of screening many samples at once through the use of trays containing multiple sample wells.

Fibrosis – Scarring and thickening of tissue, thereby weakening tissue function.

Glioma – A cancer of the glial cells that surround and support neurons.

Glomerular sclerosis – A condition in which the glomeruli – the network of blood-filtering capillaries in the kidney – become scarred and gradually lose their function.

GMP – Short for Good Manufacturing Practice, the set of standards that have been laid down by regulators such as the FDA for the production of clinical-grade pharmaceuticals. cGMP refers to 'current' Good Manufacturing Practice, since GMP standards tend to change over time.

Growth factor – A protein that stimulates cell division, differentiation and proliferation.

Heart failure – A condition where the heart is unable to pump adequate amounts of blood around the body. Heart failure often follows on from an Acute Myocardial Infarction (ie heart attack) or chronic inflammation.

Heparin – A compound occurring in the liver and other tissues which inhibits blood coagulation. Natural and synthetic heparin are used as anti-clotting drugs.

In vitro – Latin for 'in glass', referring to experiments carried out in systems outside the body, such as cells grown in culture dishes.

***in vivo* –** Experiments carried out in living organisms**.**

Ischemia – Lack of adequate blood flow to support the normal functioning of a tissue.

Knockout mice – Mice that have been genetically engineered to lack a specific gene, so that the effect of that loss of gene function can be studied.

Macrophages – White blood cells involved in the immune system's response to infection or tissue damage.

Midkine (MK or MDK) – A small heparin-binding growth factor protein that is important in embryonic development and, while not detectable in healthy adults, is highly expressed in cancer, inflammatory conditions and autoimmune disorders.

Monoclonal antibodies – Antibodies cloned from a particular cell-making antibody that is highly specific for a particular antigen. Monoclonal antibodies are increasingly used as drugs.

Myocarditis – Inflammation of the heart muscle, the myocardium.

Neuroblastoma – A cancer of the neuroblast nerve cell precursors, occurring most often in infants and young children. Neuroblastoma is rare, with only ~700 new cases in the US each year.

NETosis – A process where web-like chromatin structures known as 'neutrophil extracellular traps (NETs) are formed by neutrophils. NETs engulf and kill microbial pathogens.

Neutrophil – A white blood cell vital for innate immune system function. Neutrophils work by ingesting foreign cells and also releasing cell contents that are toxic to microbes

Non-small-cell lung cancer (NSCLC) – One of two main types of lung cancer, the other being small-cell lung carcinoma. Non-small cell lung cancer is easier to surgically remove.

pg/mL – Picograms per millilitre, a measurement of the serum concentration of rarely-occurring proteins. A picogram is a trillionth of a gram.

Phase – A stage of the clinical trialling process for a drug candidate. Phase I tests for safety in healthy controls. Phase II tests for efficacy in a small cohort of diseased individuals. Phase III tests for efficacy in a large patient group.

Regulatory T cells – T cells which turn down an immune response.

Remodelling – Permanent adaptations in cardiac tissue to compensate for loss of contractile cardiac muscle in heart failure.

Sepsis – Serious and potentially life-threatening systemic inflammation caused by severe infection.

Serum – Blood fluid from which the clotting proteins have been removed. Plasma is the clear fraction of the blood. The serum concentration of a protein relative to its normal level can be indicative of disease.

siRNA – Short for Silencing RNA, siRNA refers to a particular gene type that silences or mutes the gene expression, essentially suppressing the normal gene reaction to protect from viruses. The Nobel Prize in Physiology/Medicine for 2006 went to the Americans Andrew Fire and Craig Mello for their discovery of gene silencing.

TReg – See Regulatory T cells.

Wild-type – The natural version of a particular gene, protein, or strain.

Xenograft – An animal model of cancer in which a human tumour is grafted onto a mouse without a functioning immune system, so that the human tumour will not be rejected.

Appendix II – LYRAMID's Intellectual Property

The intellectual property behind LYRAMID's Midkine programme is currently covered by eight patent families:

Preventive for adhesion following abdominal surgery, WO/2004/078210, priority date 6 March 2003[67]. Invented by Takashi Muramatsu, Kazuhiko Ino, Hisako Muramatsu and Shuhei Torii.

- This patent application covers the use of Midkine inhibitors in preventing surgical adhesions by blunting the associated inflammation.

Composition for treating or preventing myocardial disorder or heart failure, WO/2006/062087 priority date 6 December 2004[68]. Invented by Mitsuru Horiba, Itsuo Kodama, Takashi Muramatsu and Kenji Kadomatsu.

- This patent application covers the use of Midkine protein in treating heart attack due to blocked coronary arteries and thereby preventing ischemic heart failure.

Pharmaceutical composition for vascular occlusive disease, WO/2006/126600, priority date 25 May 2005[69]. Invented by Kenji Kadomatsu, Hiroshi Banno, Yoshifumi Takei, Kimihiro Komori, Takashi Muramatsu and Sadatoshi Sakuma.

- This patent application covers the use of Midkine to treat various disease conditions in which blood vessels are in danger of blockage.

Method for treatment or prevention of disease associated with functional disorder of regulatory T cells, WO/2007/055378, priority date 14 November 2005[70]. Invented by Meisei Chikara, Akio Suzumura, Kanaiwa Ou, Matsui Takataka, Matsui Sadatoshi, Sakuma Shin, Miyagawa Masatoshi, Fujiwara Yoshikazu and Nakamura Yoshikazu.

- This patent application covers the use of Midkine antagonists in treating Multiple Sclerosis.

[67] This patent application has been granted in the US as Patent No. 8,221,758 (July 2012) and US as Patent No. 8,748,406 (June 2014) and in Europe as EP 1 607 102 (January 2017)
[68] This patent application has been granted in the US as Patent No. 9,023,799 (May 2015) and in Europe as EP 1 832 296 (August 2009).
[69] This patent application has been granted in Europe as EP 1 900 380 (March 2013).
[70] This patent application has been granted in the US as Patent No. 8,128,934 (March 2012).

Antibody recognising C-domain of Midkine, WO/2008/059616, priority date 14 November 2006[71]. Invented by Sadatoshi Sakuma, Takashi Matsui, Takashi Muramatsu, Masatoshi Hayashibara, Takanori Ito and Tsukasa Uno.

- This patent application covers antibodies to one of the two Midkine protein domains.

Antibody recognising N-domain of Midkine, WO/2012/122590, priority date 14 March 2011[72]. Invented by Sadatosi Sakuma, Maria Halasz and Darren Jones.

- This patent application covers antibodies to the other domain of Midkine protein, with the aforementioned WO/2008/059616 having covered the C-domain.

Improved Midkine antibody, WO/2016/058047, priority date 14 October 2014[73]. Invented by Maria Halasz, Darren Jones, Nico Mertens and Phillip Cunnah.

- This patent application covers the first of the humanised Midkine antibodies.

Methods of treating myocarditis and/or cardiomyopathy and reagents therefor, WO/2019/136516, priority date 9 March 2018. Invented by Maria Halasz, Darren Jones, Ulrich Grabmaier, Ludwig Weckbach, Barbara Walzog and Graham Robertson.

- This patent application covers the use of Midkine antibodies in blunting the inflammation and loss of cardiac performance in myocarditis.

Appendix III – Relevant papers

Midkine in kidney disease

Hobo et. al. (2009), *The growth factor Midkine regulates the Renin-Angiotensin System in mice*. J Clin Invest. 119:1616-25.

- The standard of care today for treating both high blood pressure and chronic kidney disease are ACE inhibitors and the angiotensin receptor blockers (ARBS) that act on the Renin-Angiotensin System. This paper shows that angiotensin and Midkine tend to operate in tandem, suggesting Midkine is an important target in kidney disease and blood pressure regulation.

Sato and Sato (2014), *Midkine in nephrogenesis, hypertension and kidney diseases*. Review Br J Pharmacol. 171:879-87.

- This review provides comprehensive coverage of the role Midkine plays in kidney disease.

Campbell et. al. (2021), *Midkine and chronic kidney disease-associated multisystem organ dysfunctions.* Nephrol Dial Transplant. 36:1577-1584.

- This paper, by A/Prof Graham Robertson (LYRAMID) and researchers at Sunshine Coast University Hospital in Queensland, provides an overview of the role of Midkine in Chronic Kidney Disease, showing in particular that Midkine levels progressively increase as renal function declines (as measured by glomerular filtration rate).

[71] This patent application has been granted in the US as Patent No. 9,163,081 (October 2015) and in Europe as EP 2 088 159 (March 2014).
[72] This patent application has been granted in the US as Patent No. 9,624,294 (April 2017) and in Europe as EP 2 686 016 (May 2019).
[73] This patent application has been granted in the US as Patent No. 10,590,192 (March 2020) and in Europe as EP 3 206 712 (December 2019).

Midkine in cardiovascular disease

Takemoto et. al. (2017), *Midkine promotes atherosclerotic plaque formation through its pro-inflammatory, angiogenic and anti-apoptotic functions in Apolipoprotein E-knockout mice*. Circ J. 82:19-27.

- This paper shows the potential of Midkine antagonists to prevent atherosclerosis.

Weckbach et. al. (2019), *Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis*. J Exp Med. 216:350-368.

- This paper describes the link between Midkine and myocarditis, which involves the presence of Neutrophil Extracellular Traps. It also demonstrated the efficacy of LYRAMID's midkine antibodies in reducing inflammation and fibrosis in heart muscle, while preserving cardiac performance as revealed by echocardiography.

Jeffrey et. al. (2021), *Serum circulating proteins from pediatric patients with dilated cardiomyopathy cause pathologic remodeling and cardiomyocyte stiffness*. JCI Insight. 6:e148637.

- This paper identifies a link between Midkine and dilated cardiomyopathy in children.

Midkine in cancer

Kishida et. al. (2013), *Midkine promotes neuroblastoma through Notch2 signaling*. Cancer Res. 73:1318-27.

- This paper shows that Midkine is important in brain cancer.

Cohen and Shachar (2014), *Midkine as a regulator of B cell survival in health and disease*. Br J Pharmacol. 171: 888–895.

- This paper considers the evidence that Midkine antagonists may be able to treat blood cancers such as Hodgkin's Lymphoma and Chronic Lymphocytic Leukaemia.

Güngör et. al. (2014), *Pancreatic cancer*, Br J Pharmacol. 171:849-58.

- This paper looks at new targets for the treatment of pancreatic cancer including Midkine.

Olmeda et. al. (2017), *Whole-body imaging of lymphovascular niches identifies pre-metastatic roles of Midkine.* Nature. 546:676-680.

- This paper, from the laboratory of Prof Maria Soengas at CNIO in Madrid, established that Midkine is pivotal to melanoma tumours ability to spread to lymph nodes and distal organs. This suggests the potential for Midkine inhibitors to prevent fatal metastasis in melanoma and other cancers.

Cerezo-Wallis et. al. (2020), *Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state*. Nat Med. 26:1865-1877.

- This paper, also from the Soengas lab, showed that melanoma was able to blunt an anti-cancer immune response in part through Midkine activity in the tumour microenvironment.

López-Valero et. al. (2020), *Midkine signaling maintains the self-renewal and tumorigenic capacity of glioma initiating cells*. Theranostics. 10:5120-5136.

- This paper shows that the cancer stem cells that promote gliomas rely in part on Midkine for their treatment resistance.

Guo et. al. (2020), *Midkine activation of CD8 + T cells establishes a neuron-immune-cancer axis responsible for low-grade glioma growth*. Nat Commun. 11):2177.

- This paper shows that gliomas are able to thrive in part because the brain's immune system is activating memory T cells to protect the glioma, and that Midkine secreted by neuronal cells plays a role in this.

Cubillos-Zapata et. al (2020), *Proangiogenic factor Midkine is increased in melanoma patients with sleep apnea and induces tumor cell proliferation*. FASEB J. 34:16179-16190.

- This paper used 360 patients in sleep studies to show a link between melanoma cancer severity and sleep apnea, with Midkine driving the cancer.

Zhang et. al. (2021), *Single-cell RNA-sequencing atlas reveals an MDK-dependent immunosuppressive environment in ErbB pathway-mutated gallbladder cancer*. J Hepatol. S0168-8278(21)00442-6. Online ahead of print.

- This paper looked at the 'transcriptome' of bladder cancer and found a link between Midkine and the blunting of an anti-cancer immune response, with tumour-infiltrating macrophages carrying a molecule called LRP1, which is one of Midkine's cell surface receptors.

Midkine in lung disease and COVID-19 infection

Misa et. al. (2017), *Involvement of Midkine in the development of pulmonary fibrosis*. Physiol Rep. 5: e13383.

- This paper shows *in vivo* how Midkine exacerbates pulmonary fibrosis in part by regulating inflammatory cell migration into the lung.

Barnes et. al. (2020), *Targeting potential drivers of COVID-19: Neutrophil extracellular traps*. J Exp Med. 217:e20200652.

- This paper shows that neutrophil extracellular traps contribute to organ damage and mortality in COVID-19.

Kinoshita et. al. (2020), *Growth factor Midkine aggravates Pulmonary Arterial Hypertension via surface nucleolin*. Sci Rep. 2020 10:10345.

- This paper explores the link between Midkine and Pulmonary Arterial Hypertension and identifies a pathway that involves Midkine as a potential therapeutic target.

Skendros et. al. (2020), *Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis*. J Clin Invest. 130:6151-6157.

- This paper further explores the link between neutrophil extracellular traps and COVID-19, as it pertains to the thromboses experienced by some patients.

Sanino et. al. (2020), *Physiology of Midkine and its potential pathophysiological role in COVID-19*, Front Physiol. 11:616552.

- This paper was the first review of the potential role of Midkine in COVID-19, covering the NETosis aspect as well as its potential involvement in viral entry.

Xu et. al. (2021), *Pulmonary Midkine inhibition ameliorates sepsis-induced lung injury*, J Transl Med. 19:91.

- This paper reports clinical evidence of the role of elevated Midkine in sepsis and how blocking Midkine reduced lung injury in a setting that is

similar to COVID-19.

Midkine in autoimmune disorders

Wang et. al. (2008), *Inhibition of Midkine alleviates Experimental Autoimmune Encephalomyelitis through the expansion of regulatory T cell population*. Proc Natl Acad Sci U S A. 105:3915-20.

- This paper showed that Midkine inhibition could work in Multiple Sclerosis, through the Treg cells that would blunt the autoimmune responses and inflammation in the CNS.

Sonobe et. al. (2012), *Midkine inhibits inducible regulatory T cell differentiation by suppressing the development of tolerogenic dendritic cells*. J Immunol. 188:2602-11.

- This paper provides further evidence on Midkine inhibition in the EAE model of Multiple Sclerosis.

Weckbach et. al. (2014), *The cytokine Midkine supports neutrophil trafficking during acute inflammation by promoting adhesion via β2 integrins (CD11/CD18).* Blood. 123:1887-96.

- This paper shows Midkine's involvement in recruiting neutrophils to sites of tissue inflammation.

Takeuchi (2014), *Midkine and Multiple Sclerosis*. Br J Pharmacol. 171:931-5.

- This review looks at earlier work on the relationship between T Reg cells and Midkine in Multiple Sclerosis.

Maruda et. al. (2017), *Growth factor Midkine promotes T Cell activation through Nuclear Factor of Activated T Cells signaling and Th1 cell differentiation in Lupus Nephritis*. Am J Pathol. 187:740-751.

- This paper shows that Midkine plays a key role in the exacerbation of the kidney condition associated with lupus.

Sollberger et. al. (2018), *Neutrophil Extracellular Traps: The biology of chromatin externalization*. Dev Cell. 44:542-553.

- This review outlines the importance of NET formation in inflammatory disease processes.

Herradon et. al. (2019), *Connecting metainflammation and neuroinflammation through the PTN-MK-RPTPβ/ζ Axis: Relevance in therapeutic development*. Front Pharmacol. 10: 377.

- This review explores how Midkine contributes to neuroinflammation.

Appendix IV – Comparable companies

We selected nine publicly-traded companies that are roughly comparable to Lyramid in terms of their stage of development and their ability to potentiate cancer immunotherapies or COVID-19 therapy. This suggests a valuation range of US$15m (base case) to US$137m (optimistic case)[74].

Altamira Therapeutics[75]. This company has been built on peptide-based technology for the delivery of RNA into cells. The company's lead programme, still preclinical, is focused on delivery of siRNA targeting KRAS, which would

[74] As at 4 January 2022.
[75] Hamilton, Bermuda, Nasdaq: CYTO, altamiratherapeutics.com.

allow reduction of expression of this oncogene in cancer cells, leading to significant inhibition of tumour growth.

BioVaxys Technology Corp[76]. This company's technology allows the creation of haptens, that is, small molecules made immunogenic by their being combined with carrier molecules. The company's lead candidate is BVX-0320, a SARS-CoV-2 vaccine candidate for which an IND has been filed.

Cocrystal Pharma[77]. This company develops antiviral drugs. Prior to COVID-19 the lead candidate was in Hepatitis C. Since 2020 the company has been emphasising protease inhibitors to COVID-19, where work is currently pre-clinical but where a pre-IND briefing package was submitted to the FDA in November 2021.

Oncorus[78]. This company develops viral immunotherapies, which a lead candidate an oncolytic Herpes Simplex Virus strain engineered to express various immunostimulatory transgenes. That candidate is now in Phase 1 various cancers as a monotherapy as well as in combination with Keytruda.

Oncotelic[79]. This immune-oncology company focused on TGF-β as its main target. The lead candidate is OT-101, an antisense drug against TGF-β2, for the treatment of solid tumours. OT-101 also has shown activity against SARS-CoV-2 where Phase 2 work has been done. The company is also working on CA4P, a 'vascular disrupting agent' that it intends to combine with Yervoy in melanoma.

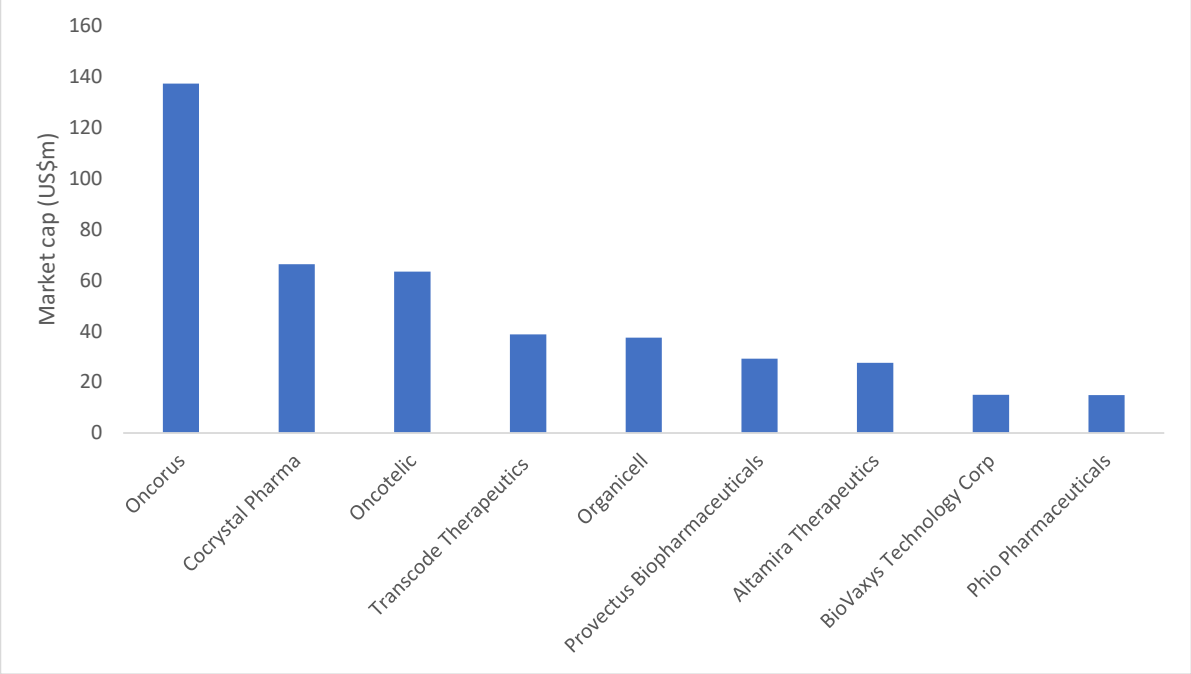
Organicell[80]. This company's technology allows exosomes to be used as RNA delivery vehicles containing numerous growth factors, cytokines, and chemokines. The lead candidate, Zofin, is being tried out first in COVID-19 and Long COVID, where it is believed to be able to suppress cytokine activation for the reduction of COVID-19 infection severity.

Phio Pharmaceuticals[81]. This company's Intasyl technology enables efficient RNAi delivery into cells. Phio is using this technology to create next-generation immuno-oncology drugs. The lead candidate, PH-762, is designed to reduce the expression of the immune checkpoint PD-1.

Provectus Biopharmaceuticals[82]. This company's PV-10 product is an injectable formulation of a xanthene dye called rose bengal disodium, known to prompt an anti-cancer immune response. The company is trying this product out in various settings including in combination with Keytruda.

Transcode Therapeutics[83]. This company develops oncology drugs across a variety of approaches in the field. The company's lead candidate, TTX-MC138, is an RNAi molecule targeting a miRNA (ie micro RNA) called miR-10b, known to be important in a number of cancers.

[76] Vancouver, BC, CSE: BIOV, biovaxys.com.
[77] Bothell, Wa., Nasdaq: COCP, cocrystalpharma.com.
[78] Cambridge, Ma., Nasdaq: ONCR, oncorus.com.
[79] Agoura Hills, Ca, OTCQB: OTLC, oncotelic.com.
[80] Miami, Fl., OTCQB: OCEL, organicell.com.
[81] Marlborough, Ma., Nasdaq: PHIO, phiopharma.com.
[82] Knoxville, Tn., OTCBC: PVCT, provectusbio.com.
[83] Worcester, Ma., Nasdaq: RNAZ, transcodetherapeutics.com.



Appendix V – The story so far

Cellmid did over a decade of pioneering work on Midkine

Cellmid worked on Midkine in a variety of disease conditions. Over the twelve year period in which Cellmid was focused on Midkine, the company developed programmes in cancer, autoimmune disorders as well as chronic inflammatory renal and cardiovascular disease.

Cellmid developed a variety of Midkine-antibodies. The company acquired a large portfolio of method and composition of matter patents covering the use of Midkine and Midkine inhibitors in a range of disease indications. It also got in excess of 120 proprietary antibodies targeting Midkine, and seven grams of purified Midkine protein. Cellmid subsequently humanised four antibodies in order to progress to clinical development. The first humanised Midkine antibody was generated in 2011.

Cellmid worked in four basic programmes for Midkine:

- **Cancer**. Cellmid developed anti-tumour antibodies and had evidence by 2013 that its Midkine antibodies could function well in terms of slowing primary tumour growth and tumour metastasis as well as restricting angiogenesis[84]. The following year Cellmid selected a lead candidate monoclonal antibody called IP14 that was humanised as CAB102. In studies involving human tumours implanted into mice CAB102 shrunk Non-Small Cell Lung Cancer tumours by 50% over 21 days in conjunction with carboplatin ($p<0.01$ versus carboplatin only)[85]. By 2016 the company had evidence that its antibodies worked in brain cancer[86], a finding that was subsequently confirmed in 2020 in studies showing reduced glioma cell growth[8].

[84] See the Cellmid market release dated 3 October 2013 and headlined '*Midkine antibodies effective in cancer*'.
[85] See the Cellmid market release dated 7 May 2014 and headlined '*Key milestone completed: Cellmid lead antibody selected for clinical trials*'.
[86] See the Cellmid market release dated 5 October 2016 and headlined '*Cellmid's Midkine antibodies show anti-tumour activity in brain cancer*'.

- **Cardiovascular disease.** Cellmid was very interested in the potential of Midkine antibodies to protect the heart in autoimmune myocarditis, a form of chronic inflammatory heart failure [87]. In 2019 Cellmid's Midkine antibodies were shown to prevent heart muscle damage and fibrosis from inflammation in myocarditis[88]. Echocardiography revealed that heart performance was maintained with cardiac output normalised following treatment with the Midkine antibodies. A key finding from this study was that the antibodies reduced the recruitment of neutrophils into inflamed heart muscle and also blocked neutrophil activation via an inflammatory process called NETosis. These findings have major clinical significance for two reasons. Firstly, heart failure due to chronic inflammation is emerging as a greater health problem than acute heart attacks due to blocked coronary arteries. Secondly, NETosis contributes to the pathology of many diseases, including severe COVID-19.
- **Autoimmunity.** Cellmid reported evidence in 2010 that its antibodies raised Treg cell counts in autoimmune models of rheumatoid arthritis and Multiple Sclerosis. Normalising Treg cells blocked autoimmune cell responses and reduced disease severity in the murine EAE (Experimental Autoimmune Encephalitis) model of MS[89].
- **Kidney disease**. Cellmid reported in 2013 that Midkine antibodies reduced kidney damage in mouse models of diabetic nephropathy[90]. By 2018 there was *in vivo* evidence that the murine, as well as the humanised CAB102 antibodies reduced renal inflammation and fibrosis while preserving kidney function in a rare disorder called Focal Segmental GlomuleroSclerosis (FSGS)[91].

Cellmid developed a Midkine diagnostic assay, the MK-ELISA kit, that was validated, GMP manufactured and CE marked and marketed by 2011[92]. Combined with a patent family covering Midkine as an early biomarker of solid tumours, this opened up the potential of a 'companion diagnostic' to go with future therapeutic products for oncology. Having IP coverage for an important cancer biomarker, in combination with a validated proprietary diagnostic kit for Midkine, also highlights the potential for the development of personalised medicine approaches to cancer treatment based on Midkine.

Cellmid chose to exit Midkine mainly because of the costs of development. Drug development programs, especially antibody drugs that had previously been the focus for Cellmid, are expensive, costing around US$1bn per approved drug across the entire preclinical and clinical pathway and entry to market[93]. As Cellmid began to build its portfolio of consumer health products, it made a strategic decision to pivot away from drug development and focus on the market launch of its hair loss and anti-aging products. To reflect this change Cellmid changed its name in late 2021 to Anagenics, ASX: AN1. Having spent more than a decade on research and development, and uncovering both the biology and therapeutic potential of targeting Midkine, Cellmid/Anagenics retains a strong interest in the success of commercialising the Midkine intellectual property through its license to LYRAMID. In turn, LYRAMID can take advantage of the potential upside of the significant knowledge and asset base accumulated through this period.

[87] See Lopez-Valero et.al. (2020) *Midkine signaling maintains the self-renewal and tumorigenic capacity of glioma initiating cells.* Theranostics 10:5120. .
[88] See Weckbach et. al. (2019), *Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis*. J Exp Med. 216:350-368.
[89] See the Cellmid market release dated 19 August 2010 and headlined *'Preclinical study shows reduction in disease severity'*.
[90] See the Cellmid market release dated 23 January 2013 and headlined *'Cellmid records positive data in Midkine antibody study in kidney disease'*.
[91] See the Cellmid market release dated 12 September 2018 and headlined '*Cellmid's lead antibody effective in rare chronic kidney disease'*.
[92] See the Cellmid market release dated 3 November 2010 and headlined '*Cellmid launches Midkine blood test'*.
[93] See Wouters et. al. (2020), *Estimated Research and Development investment needed to bring a new medicine to market, 2009-2018.* JAMA . 2020 Mar 3;323(9):844-853.

Appendix VI – Risks related to LYRAMID and Roquefort Therapeutics

Risks specific to LYRAMID. We see four major risks for LYRAMID as a company and as a listed stock:

- **Timing risk.** There is the risk that the progression of LYRAMID science into the clinic may take longer than expected.
- **Regulatory risk**. There is the risk that the FDA and other regulators may decline to approve LYRAMID products, even if LYRAMID considers the data submitted to be adequate.
- **Commercial risk**. There is the risk that LYRAMID may fail to find commercial partners.
- **Uptake risk**. LYRAMID products may fail to find significant usage in COVID-19 as other therapies come onto the market between now and the end of LYRAMID's clinical development.

Risks related to pre-revenue Life Science companies in general.

The stocks of biotechnology and medical device companies without revenue streams from product sales or ongoing service revenue should always be regarded as speculative in character.

Since most biotechnology and medical device companies listed on the AIM fit this description, the 'term' speculative can reasonably be applied to the entire sector.

The fact that the intellectual property base of most biotechnology and medical device lies in science not generally regarded as accessible to the layman adds further to the riskiness with which the sector ought to be regarded.

Caveat emptor. Investors are advised to be cognisant of the abovementioned specific and general risks before buying any the stock of any biotechnology and medical device stock mentioned on this report, including LYRAMID or Roquefort Therapeutics.

Appendix VII – Analyst Qualifications

Stuart Roberts, lead analyst on this report, has been covering the Life Sciences sector as an analyst since 2002.

- Stuart obtained a Master of Applied Finance and Investment from the Securities Institute of Australia in 2002. Previously, from the Securities Institute of Australia, he obtained a Certificate of Financial Markets (1994) and a Graduate Diploma in Finance and Investment (1999).
- Stuart joined Southern Cross Equities as an equities analyst in April 2001. From February 2002 to July 2013, his research specialty at Southern Cross Equities and its acquirer, Bell Potter Securities, was Healthcare and Biotechnology. During this time, he covered a variety of established healthcare companies such as CSL, Cochlear and Resmed, as well as numerous emerging companies. Stuart was a Healthcare and Biotechnology analyst at Baillieu Holst from October 2013 to January 2015.
- After 15 months in 2015 and 2016 doing Investor Relations for two ASX listed cancer drug developers, Stuart founded NDF Research in May 2016 to provide issuer-sponsored equity research on ASX-listed Life Science companies.
- In July 2016, with Marc Kennis, Stuart co-founded Pitt Street Research Pty Ltd, which provides issuer-sponsored research on ASX-listed companies across the entire market, including Life Science companies.

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