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The rôle of serendipity in biotechnology start-up companies — two case studies

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Abstract

The process of starting and building a biotechnology-based company in Australia in the 2020s is now well documented and supported by a number of programs designed to support founders on their start-up "journey." However, what is often under emphasised in these programs is the importance of timing and serendipity, or luck, in determining the success of the start-up company. In this article, using the story of the founding of two companies that the author has personally been involved in their foundation, the importance of these two elements — timing and serendipity — is demonstrated.

Introduction

In Boston-based biotech guru Peter Kolchinsky's excellent handbook on establishing and running an early-stage biotechnology company, *The Entrepreneur's Guide to Biotech Start-Ups*, 4th Ed.,[1] he sets out the key ingredients needed to maximise the chances of successful growth of new biotechnology companies. These include: evaluating the commerciality of the idea; writing the business plan; finding the right people; protecting the intellectual property, and so on. It really is a valuable guide that should be on every biotech entrepreneur's reading list.

But what is lacking is any mention of serendipity (the word does not appear anywhere in the document), luck, timing (in terms of being in the right place at the right time), or of resilience.

I have been involved in founding two biotechnology companies, and of leading several other biotechnology start-ups. And in almost every case, the companies would have remained a mere idea, had it not been for a fair slice of serendipity in the discovery phase, and being the right time for the idea to take root.

In 2022 I accepted the role of Entrepreneur-in-Residence at UNSW's 10X Founders Program, mentoring the next generation of biotech start-up founders, and this caused me to contemplate the key factors that are critical to the success of such ventures.

This essay explores the role of serendipity, or luck, in the founding of two Australian biotechnology companies, proving the old adage "fortune favours the prepared mind."[2]

BCAL Diagnostics

BCAL Diagnostics Limited (BCAL) listed on the Australian Securities Exchange in

1 Available at https://www.evelexa.com

2 During an 1854 lecture at the University of Lille, French microbiologist and chemist Louis Pasteur said, "le hasard ne favorise que les esprits préparés." Gibbons (2013) refers to other cases of chance discoveries in science and biology [Ed.]

2021 (ASX:BDX). The company is developing a blood test for breast cancer. This is the story of its origins.

In 1999 Australian physicist Professor Veronica James and colleagues reported that synchrotron X-ray diffraction patterns of hair from individuals with breast cancer differed from those of healthy subjects (James et al., 1999). They reported that the patterns of hair from cancer patients contained a ring of comparatively low intensity which was superimposed on the normal α-keratin pattern obtained from healthy control subjects. This was followed over the next six years by further publications from Professor James and colleagues extending the results (e.g. Meyer & James, 2001).

After the initial excitement, the finding soon became controversial as several groups independent of Professor James failed to replicate the original results. The inventor responded by publishing technical explanations for their replication failures. In 2005, Professor James and other scientists reported on the results of 503 blinded hair-sample analyses and demonstrated a sensitivity of 100% (no false negatives) and a specificity of 86% (14% false positives by comparison to mammography) for breast cancer (James et al., 2005).

A company, Fermiscan Limited, was formed in 2006 to develop the "hair test for breast cancer," as the discovery became known, and to commercialise it. The company was back-door listed into a former mining company shell. The name paid homage to Enrico Fermi, a Nobel Prize-winning particle physicist who was involved, amongst a range of other achievements, with the high-speed particle accelerator in Illinois, the FermiLab. The X-rays used to examine the hairs for the tell-tale breast-cancer ring needed to be generated from a synchrotron, a high-speed particle accelerator. I was appointed Chief Scientist of Fermiscan. Coincidentally, my PhD research in the 1980s was focussed on understanding the keratin structure of the wool fibre, so, perhaps serendipitously, it could be assumed that I had a very appropriate background to develop the technology.

In January 2008, Gary Corino (who had worked with Professor James in the early days of the research) and I reported the results of synchrotron-generated X-ray diffraction analysis of samples from women whose breast-cancer status was known (Carino & French, 2008). In the study, 39 hair samples were examined. Nineteen hair samples were collected from women presenting at a radiology clinic, 14 positive controls (samples from women known to be diagnosed with breast cancer) and six samples from women assumed negative by mammography, were analysed. Synchrotron-based X-ray experiments were carried out at the Advanced Photon Source at the Argonne National Laboratory, outside of Chicago, USA. Analyses were conducted on two beamlines which had been previously used for studies on detection of cancer by hair diffraction and therefore offered the greatest opportunity for a successful replication of the original finding. Diffraction images were analysed using two different image-processing programs.

We reported that we were able to successfully and consistently generate the basic α-keratin pattern in hair, which was a primary requirement of the process, and more importantly, that we were able to identify the circular feature in the pattern that correlated with the presence of breast cancer in 13 of the 14 positive controls at

the defined spacing. In this small study, we had confirmed the existence of a correlation between an altered X-ray diffraction pattern of hair and the presence of breast cancer. However, we reported one subject with invasive cancer whose hair failed to produce a diffraction ring in the zone of interest. In addition, two of the samples were classified as indeterminate due to the presence of interfering features. This meant that our specificity and sensitivity were both less than 100%.

Having independently confirmed that there was an association between the presence of breast cancer and the ring in X-ray diffraction images of hair, I started to investigate the origin of the circular feature in the X-ray diffraction patterns. X-ray diffraction relies on high-speed particles being fired at the sample, and bouncing off the molecules that comprise that structure. When they bounce off, they will sometimes interact with other deflected particles, thus causing a diffraction pattern. The harder the molecules that they hit, the more they will bounce, and the resulting diffraction patterns are mainly ordered arrays of spots. This is typical of a crystal structure, as can be seen for example in crystals of sodium chloride (see for example Kemp & Alcock, 2017).

X-ray diffraction was used by Rosalind Franklin in the 1950s to try to understand the structure of DNA. Known as "Photo 51," "probably the most famous XRD (X-ray diffraction) photograph of the 20th century" (Kemp & Alcock, 2017) was taken by Rosalind Franklin's PhD student, Raymond Gosling in 1952, at King's College, London.

In this image there are no spots, but there are regular repeating elements indicating that DNA is a highly ordered oriented fibre rather than a single crystal. This was key information utilised by James Watson and Francis Crick for determining that DNA forms a double helix (Watson & Crick, 1953).[3]

X-ray diffraction of hair had been conducted for many years (even prior to the use of synchrotron-derived X-rays) (Astbury & Street, 1932), and the patterns indicate that hair is a semi-ordered structure, with several short arcs being the predominant feature.

By contrast, if a substance has no order or structure, what does the X-ray diffraction pattern look like? X-ray diffraction patterns of amorphous water are perfect rings (Kim et al., 2015).

At the stage when Fermiscan was formed, no-one had any good explanation for what was giving rise to the distinctive circular feature in hair from women with breast cancer.

A possible solution presented itself by chance, in March 2008, when I and other staff of Fermiscan visited the Diamond synchrotron in Oxford, UK. I was accompanied by a young laboratory technician, a recent BSc (Hons) graduate of Sydney University, Dharmica Mistry, who had been hired by the company to load patients' hairs onto special holders for exposure to synchrotron-derived X-ray beams. We met Gary Corino in the laboratory attached to the facility the day before we were due to test-run a number of hair samples as part of the clinical study that had been established by the company in order to test the accuracy of the technology. Gary had been working on the SAXS beam line in the synchrotron in Chicago, and in the course of his work, he had run

3 Famously, for discovering the structure of DNA, Crick and Watson shared a Nobel Prize with Franklin's colleague, Maurice Wilkins, but not Rosalind Franklin. [Ed.]

some "negative control" hairs, from women with no history of breast cancer, including Dharmica. When Dharmica and I met Gary in Oxford, the conversation proceeded as follows:

Gary Corino: "Dharmica, what do you do to your hair?"

Dharmica Mistry: "Nothing special, why?"

GC: "Well, when I run your hair as a negative control, I see a clear ring in the right position for breast cancer."

DM: "Oh! I am only 22, and there is no history of breast cancer in my family. It must be a false positive."

GC: "Well, that's why I asked what you do to your hair when you wash it."

DM: "Nothing. I just wash it with shampoo, and some conditioner, that's all."

GC: "Nothing else?"

DM: "No … Oh, wait. Sometimes I spray olive oil on it to make it shiny."

There was a pause. I knew that olive oil is very similar to many human fatty acids (lipids). In fact, as a hobby, I was a small olive oil producer myself. Lipids are amorphous substances. I immediately suggested that the breast cancer ring was due to lipids from the tumour accumulating in the hair fibre. There was some discussion about this idea, and it was agreed that we should test the idea as soon as we could.

We couldn't do anything about it at the time, but when Dharmica and I returned to Sydney, we discussed how we could test the idea that the circular feature was due to a lipid (or lipids) incorporated into the hair fibre. Later that year, we travelled to the Australian synchrotron in Melbourne, armed with several hundred hair samples from the Fermiscan clinical trial to analyse on the SAXS beam line, as well as a bottle of acetone and a bottle of olive oil. We had booked the beam line for an all-night session, as the process of loading the hairs into the beam line, scanning them with the X-rays and collecting several images per hair was quite time consuming.

When we had finished running the samples for the clinical trial, at around 3 am Dharmica suggested we run the lipid test experiment. Being much older, I was feeling exhausted, so she offered to do the work. First, she took a hair sample that we had previously analysed in the beam line and had found no ring in the diffraction pattern. She soaked it in olive oil, dried it, and then loaded it into the beam line. Sure enough, on the diffraction pattern we could clearly see a circular feature in the correct location for the putative breast-cancer feature. Next, she took a hair that had a clear breast cancer circular feature and soaked it in acetone. She then dried it and loaded it into the beam line. The previous circular feature had disappeared, but the other features were still intact, indicating that the circular feature had been removed by the acetone, a solvent that dissolves lipids. She repeated this on other hairs and confirmed the result. We were convinced that the breast-cancer-associated circular feature in the X-ray diffraction patterns was most probably caused by one or more lipids being incorporated into the hair fibre as a result of the cancer being present.

There was one more test that we wanted to conduct. After our Melbourne experiments, we found a paper by Bertrand et al. (2003) that demonstrated that treatment of hair with lead nitrate or lead acetate enhanced lipid features observed using synchrotron X-ray diffraction. They concluded that lead fixation could be a powerful tool to evaluate the lipid organisation in human hair.

We therefore wanted to see if treatment of hairs that contained the breast-cancer-associated ring with lead nitrate would enhance the feature when subjected to X-ray diffraction.

Dharmica travelled to the Australian synchrotron in Melbourne to test that in 2009. Mr Joseph Haklani was based in Melbourne at that stage, and he and Dharmica ran the experiment and confirmed that the breast-cancer ring was indeed amplified by treatment of hairs with lead nitrate. We were therefore convinced that the circular features first identified by Professor Veronica James in hairs from patients with breast cancer resulted from incorporation of lipids into the hair from the cancer. These findings were published in 2012 (Mistry et al., 2012).

Prior to publication of the 2012 paper, we drafted a patent application based on this finding, and Dharmica commenced a PhD to determine the nature of the lipids. Unfortunately, shortly after she commenced her PhD, the company went into administration, and the project ceased.

A few months later, a consortium was formed (of which I was a member) to acquire the patents and a new company was formed — SBC Research — and Dharmica was hired to continue her PhD project on investigating lipids in hair and serum, and their association with breast cancer. At that stage she and I were focussed on isolating lipids from serum, as we hypothesised that the tumours were releasing a cancer-associated phospholipid into the circulation and it was being taken up by the hair follicles and incorporated into the fibre. However, in the course of her project, we did not find as strong a correlation as we were expecting. Initially we thought this might have been due to the complexity of lipid extraction and analysis (a mass spectrometer is needed), and of the vast number of lipid species that were obtained.

Towards the end of her project there was a breakthrough. Dharmica came across a (then) recent (2011) patent that was available for licensing from the University of Louisville entitled "Methods for detecting cancer" with the following abstract:

> Methods to determine the absence or presence of one or more cancer types in an animal are disclosed herein … Amounts of lipids in a sample (e.g., a bodily fluid or treatment thereof) … are used with a predictive model to make the determination. The lipid amounts can be measured … using mass spectrometry …

Reading further, suddenly it was clear that Dharmica and I were on the right track, but were lacking one key piece of information: the lipids were not free-floating in the bloodstream as we had supposed. The inventors stated in the patent application that they were derived from "lipid microvesicles." Microvesicles are tiny particles shed from most cells, including cancer cells. One of the best-known sub-groups of microvesicles are exosomes. Exosomes are 30–120 nm particles that contain DNA, RNA and proteins enclosed within a lipid membrane. Whilst exosomes had been identified since the 1980s, by 2011 exosome science was still fairly nascent. In 2011 there were only 333 papers published on the subject of exosomes. By 2021 this had grown to 5,073 (Source: PubMed). In the earlier years, microvesicle research was difficult, as it was hard to distinguish the small vesicles from cell debris or apoptotic bodies, and long and complex ultracentrifugation techniques were required to isolate them. This was predominantly still the case in 2011.

SBC Research negotiated with the University of Louisville to license the patent, as this was clearly the vital missing link in Dharmica's and my joint hypothesis (i.e. that lipids shed from tumours were a key unique biomarker for breast cancer). The key difference was that the lipids were not free-floating in the bloodstream as we had assumed; rather, they were associated with microvesicles! SBC Research successfully negotiated the licence, and the company focussed on exploring the specific vesicle-associated lipids that could be used as markers of breast cancer.

SBC Research changed its name to BCAL Diagnostics — BCAL standing for Breast Cancer-Associated Lipids — and ultimately listed on the ASX in 2021.

There were two key serendipitous breakthrough moments critical for BCAL's progression. The first was employing a research assistant who used olive oil on her hair, and the second was the parallel work and timeliness of the patent from the University of Louisville. If we had taken longer to understand the lipid basis of the circular feature in the X-ray diffraction pattern, we would have missed the window of opportunity to license the patent and thus understand the key association with extracellular vesicles and breast-cancer lipids.

Cryosite Pty Ltd

Several years earlier, in 1997, I was employed as Manager of the Centre for Immunology at St Vincent's Hospital in Sydney. St Vincent's had been at the epicentre of the HIV/AIDS epidemic in the 1980s and 1990s, and researchers and clinicians had cared for a large number of AIDS patients. The Centre for Immunology had accumulated a very large archive of patients' serum samples, that formed an invaluable resource to understand the disease and its progression. They were stored in over twenty –80° C freezers in the Centre for Immunology's building. They were individually fitted with alarms that fed back to the hospital switchboard for 24/7 monitoring. One night, at 3 am, I was roused from my sleep by a call from the hospital informing me that one of the freezers' alarms had triggered. I immediately got dressed and drove to Darlinghurst to address the problem (by moving the samples to an empty, back-up, freezer). On the way home, I thought that there must be a better way of managing such a collection. So, the next day I started to call around various cold-storage companies to find out whether I could outsource the storage and monitoring of ultracold storage of biological samples. One call I made was typical of all of them, but this one stood out for the no-nonsense approach:

PF: "Hello, I am wondering if you have facilities to store several thousand samples at –80° C in your facility?"

The manager of the cold storage facility: "Sorry, we only go down to –40° C here. What type of samples are we talking about?"

PF: "HIV-positive serum (blood) samples."

Manager: "We store food in our freezers. Our clients are not going to want your blood samples anywhere near their food!"

PF: "Do you know of anyone who can do this type of storage?"

Manager: "No."

In thinking about this, I wondered whether there might be a market opportunity here. And so, after talking to some colleagues with a commercial background, the concept — and ultimately the company Cryosite Pty Limited — was born, and listed on the ASX in 2000. Cryosite Limited still

exists today (ASX:CTE), having expanded and changed its business model to include stem-cell storage and clinical-trial logistics.

If the freezer alarm had triggered during working hours, I might have been less motivated to find an outsourced solution. Again, as in the case study above, serendipity and timing were critical to the initial founding of the company.

Conclusion

The above case studies clearly demonstrate that, without unplanned or unforeseen occurrences, the key breakthroughs leading to company formation and on-going success would not have happened. That chance favours a prepared mind is a truism. In the case of BCAL, I was aware of the similarity between human lipids and olive oil from my olive-oil production experience. This helped me to quickly make the link between lipids from olive oil and breast-cancer-derived lipids. In the case of Cryosite, I had been undertaking an MBA, so I was aware of the elements of establishing a business based on market need, so when the market need presented itself, via the early morning call, I could see the business opportunity.

Of course, luck is not the only element of success, but it can play a key part in company formation or advancement. The other elements, identified by Kolchinsky, are obviously very important as well. Luck may not be a strategy, but it can certainly help in the high-risk field of biotechnology company establishment and growth.

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