Institution: University of Cambridge		
Unit of Assessment: 5		
Title of case study: Revolutionising drug discovery using a fragment-based approach		
Period when the underpinning research was undertaken: 2000 – 2016		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Sir Tom Blundell	**Role(s) (e.g. job title):** Professor of Biochemistry; Voluntary Director of Research	**Period(s) employed by submitting HEI:** 01 Oct 1995 - date
Period when the claimed impact occurred: Aug 2013 – Jul 2020		
Is this case study continued from a case study submitted in 2014? Y		

1. Summary of the impact

The application of X-ray crystallography to the screening of small molecule fragments for protein binding, initiated in the Departments of Biochemistry and Chemistry at the University of Cambridge, led to the structure-guided drug discovery approach implemented at the company Astex. Astex has used this methodology to discover two new highly successful anti-cancer drugs that appeared on the market in 2017 (Kisqali for breast cancer) and 2019 (Balversa for urothelial cancer). The fragment-based approach is now used by most major pharmaceutical companies, and over 40 compounds discovered using fragments have reached clinical trials. Astex currently employs 140 people and has a turnover of GBP 72,000,000.

2. Underpinning research

Tom Blundell, in collaboration with Chris Abell in the Department of Chemistry and Harren Jhoti from the company Astex, pioneered the use of small fragments in drug discovery. Their work at the University of Cambridge using X-ray crystallography to screen small molecule fragments binding to proteins led to Fragment-Based Drug Discovery (FBDD), which has revolutionised drug discovery and is now used throughout the pharmaceutical industry.

Fragment-based drug discovery (FBDD)
FBDD is a method for finding lead compounds as part of the drug discovery process. In traditional high throughput screening (HTS), molecules with high affinity binding are sought, requiring libraries of up to a million large drug-like molecules; in FBDD, much smaller molecules, such as small, organic molecules with molecular weight of < 300 Da, are identified that may bind only weakly to the drug target. These chemical fragments can then be elaborated or combined to produce larger molecules with higher affinity. This method means that a library of only hundreds of small fragments is required and results in a more efficient method than HTS.

The team realized that high-throughput X-ray crystallography could provide a powerful method for determining high-resolution three-dimensional structures of fragments bound to protein targets by screening multiple crystals of target proteins. This allows identification of those small molecules that bind, and their position and orientation at the binding site can be defined. This had the added advantage of identifying which other molecules might bind nearby, thus facilitating combining the small molecules to make higher affinity compounds. This idea was explored by soaking single protein crystals in concentrated solutions of fragments to initiate binding, then demonstrating that chemical elaboration of the fragment

could produce drug-like molecules. By March 2001, the proof-of-principle experiments were in place, and the use of X-ray crystallography to carry out fragment screening for drug discovery was described in two highly influential papers published in 2002 [R1, R2]. Based on these results, GBP 28 million in investments was secured in 2001 and Astex established their first research laboratory on the Cambridge Science Park. Blundell and Abell have collaborated closely with Astex over many years, working on solutions to challenges in the practical implementation of X-ray based FBDD.

Fragment linking
The early work in 2000/2001 described above considered fragment growing in which fragments are elaborated, guided by structure, to pick up additional interactions with the protein main-chain and sidechains in the binding site, but then focused more on fragment linking, when different fragment binding sites are co-located in the same pocket of the protein structure. As part of a Blundell and Abell collaboration targeting tuberculosis using FBDD, a comparative analysis in 2009 explored the relative merits of fragment linking and fragment growing in the rapid development of enzyme inhibitors [R3]. X-ray crystallography methods were complemented by a nuclear magnetic resonance (NMR) approach in 2010 to identify the most promising fragment linking strategy exploiting inter-fragment Nuclear Overhauser Effects (NOEs) [R4].

Targeting protein-protein interactions
In 2013, the Blundell lab defined structures of complex multi-protein systems; these proved to have small pockets at the interfaces, usually in shallow grooves. With the Abell group, they showed that these could be targeted by fragment-based screening, even though these interactions were previously considered undruggable [R5]. This has encouraged others, including Astex, to look at interfaces, in addition to enzyme active sites, as possible drug targets.

Hotspot mapping
In 2016, the Blundell lab developed a computational approach that samples atomic hotspots (locations that are crucial for ligand binding) with three simple molecular fragment probes comprising aromatic rings with substitutions by a hydrogen-bond donor, by a hydrogen-bond acceptor or by a lipophilic group. The resulting fragment hotspot maps specifically highlight fragment-binding sites within potential pharmacophores [R6]. They provide a visual guide within the binding pocket, allowing medicinal chemists to grow the molecule to make further interactions and alerting them to suboptimal interactions within the original hit.
The research undertaken by Blundell, Abell and Astex has led to significant commercial impact, resulting in new drugs being developed and approved for treatment of two types of cancer, as explained further in section 4.

3. References to the research

R1. **Blundell, T. L**.; Abell, C.; Cleasby, A.; Hartshorn, M. J.; Tickle, I. J.; Parasini, E.; Jhoti, H. High-throughput X-ray Crystallography for Drug Discovery. Drug Design: Cutting Edge Approaches. Darren Flower, Ed. Royal Society Chemistry 2002, 53-59.

R2. **Blundell, T. L**.; Jhoti, H.; Abell, C. High-throughput Crystallography for Lead Discovery in Drug Design. *Nature Reviews Drug Discovery* 2002, *1*, 45-54.

R3. Hung, A. W.; Silvestre, H. L.; Wen, S.; Ciulli, A.; **Blundell, T. L**.; Abell, C. Application of Fragment Growing and Fragment Linking to the Discovery of Novel Inhibitors of Mycobacterium Tuberculosis Pantothenate Synthetase. *Angew. Chem. Int. Ed.* 2009, *48*, 8452-8456.

R4. Sledz, P.; Silvestre, H. L.; Hung, A. W.; Ciulli, A.; **Blundell, T**. **L**.; Abell, C. Optimisation of the Interligand Overhauser Effect for Fragment Linking: Application to Inhibitor Discovery

against Mycobacterium Tuberculosis Pantothenate Synthetase. *J. Am. Chem. Soc.* 2010, *132*, 4544-4545.

R5. Scott DE, Ehebauer MT, Pukala T, Marsh M, **Blundell TL**, Venkitaraman AR, Abell C, Hyvönen M (2013) Using a Fragment-Based Approach to Target Protein–Protein Interactions. Chembiochem. 2013 Feb 11;14(3):332-42.

R6. Radoux C.J.; Olsson, T.S.G.; Pitt, W.R.; Groom, C.R.; **Blundell, T.L.** Identifying Interactions that Determine Fragment Binding at Protein Hotspots J. Med. Chem. 2016, 59, 4314–4325

All research outputs have been published in peer-review journals.

Grants

- EU FP5: Protein-protein interactions : biophysical and computational characterisation of growth factor-receptor binding and application to drug design (2002-2004), PI: Tom Blundell, GBP1,562,090.00
- BBSRC: Mechanistic and structural studies on chorismate-utilising enzymes (2005-2008), PI: Chris Abell, GBP266,529
- Wellcome Trust: Senior Investigator Award "Structural and functional studies of regulatory assemblies and macromolecular machines" (2005-2011), PI: Prof Ben Luisi GBP2,008,415.00
- BBSRC: Development of fragment-based approaches to build chemical tools for biology (2006 -2009), PI: Chris Abell, GBP216,624.99
- BBSRC: Structural studies on enzymes involved in the formation of salicylate and p-aminobenzoate (2006 -2009), PI: Chris Abell, GBP226,075.99

4. Details of the impact

Adoption of FBDD by the pharmaceutical industry

The structure-guided FBDD approach, pioneered by Blundell, Abell and Jhoti through the foundation of Astex, has led to the widespread adoption of this technology throughout the pharmaceutical industry, changing the way that drug discovery is carried out. The approach has become so embedded in the industry that there are now dedicated structural FBDD groups within most major pharmaceutical companies [E1], including AstraZeneca, Merck, Novartis, Pfizer and Abbvie [E2]. By 2018, over 40 compounds were or had been in clinical development due to the uptake of this technology [E2].

Astex

The company (named sequentially Astex Technology, Astex Therapeutics and now Astex Pharmaceuticals) was originally founded by Professors Blundell and Abell and Dr Jhoti to exploit FBDD using X-ray crystallography. Since its foundation in 1999, Astex has positioned itself as a world-leading pharmaceutical company in FBDD, and in September 2013 this was demonstrated when it was acquired by Otsuka Pharmaceutical for USD 866,000,000 [E3]. The company employs 140 people and in 2018 had a turnover of GBP 72,200,000 [E1]. Blundell remains on the Astex Board which ensures a continuing dynamic exchange of ideas between the Blundell laboratory in the Department of Biochemistry and Astex [E4]. The original 2002 papers set the manifesto for how Astex would implement FBDD using X-ray crystallography [R1, R2]. Astex continues to be a world-leader in FBDD with a strong proprietary clinical pipeline targeting cancer and diseases of the Central Nervous System. This has led to three drugs being taken to market, as detailed below, along with another five currently in clinical trials [E5], one of which (ASTX660) was granted Orphan Drug Designation by the FDA in 2020 for the treatment of T-cell Lymphomas [E6].

Drugs in the market

Kisqali®

In 2017, Astex's first drug, Kisqali®, also called Ribociclib or LEE011, was launched by Novartis [E7]. Kisqali® is an inhibitor of the cyclin-dependent kinases CDK4/6 and is used in combination with another drug, Letrozole, as a first-line treatment of an advanced form of breast cancer called hormone receptor positive, human epidermal growth factor receptor-2 negative, HR+/HER2-. Kisqali® was discovered as part of a collaboration between Astex and Novartis in 2005, and a key step was the determination of the crystal structure of CDK4 by scientists at Astex [E7]. The compound was taken through preclinical and clinical development by Novartis, and gained approval for commercialization in 75 countries [E7] including approval by the US Food and Drug Administration (FDA) (March 2017) and by the European Medicines Agency (EMA) (August 2017) [E7]. Sales of Kisqali® for the 2019 financial year were USD 480,000,000 [E8. In June 2019 Novartis published clinical trial data showing a significant increase in survival rate for patients treated with Kisqali® and Letrozole. Survival rates at 42 months were 70.2% for the Kisqali® combination therapy compared to 46.0% for endocrine therapy alone [E9]. The Chief Executive at Breast Cancer Care and Breast Cancer Now said "... this combination treatment can offer patients invaluable extra time before their disease progresses – time to live well and continue with day-to-day activities such as working for as long as possible. This option could also help delay the need for chemotherapy and the debilitating side effects it can bring" [E10].

Balversa™

Another Astex clinical candidate, Balversa™, (also called Erdafitinib) was so successful in Phase 2 clinical trials that it was granted Breakthrough Therapy Designation by the US FDA in 2018 for the treatment of metastatic urothelial cancer, the sixth most common type of cancer in the USA [E7, E11]. This designation led to rapid review and approval of the drug for marketing in the USA in April 2019 [E7]. Balversa™ was discovered using FBDD by Astex as part of a collaboration with Janssen which commenced in June 2008 to identify novel, small molecule inhibitors of Fibroblast Growth Factor Receptor (FGFR) kinase. Janssen was responsible for the clinical development and commercialization of Balversa™ [E11], which is the first personalized treatment targeting susceptible FGFR genetic alterations for patients with metastatic bladder cancer [E7].

The work on FBDD by Professors Blundell and Abell and Dr Jhoti has created a shift in the way pharmaceutical companies discover new lead compounds for drug discovery. It has led to the creation of jobs including 140 at Astex alone, generated a large contribution to the economy, including USD 866,000,000 for the sale of Astex and USD 480,000,000 for Kisqali sales in 2019-2020, and the approval in 75 countries of three drugs that are creating a positive impact on the health and wellbeing of patients with breast and urothelial cancer and leukemia.

5. Sources to corroborate the impact

E1. Testimonial from Astex
E2. Practical Fragments – Blog for Fragment-based Drug Design Practitioners 06.10.2018 "Fragments in the clinic: 2018 edition"
E3. Nature Biotechnology - Otsuka Pharmaceutical Acquisition 08.11.2013 "Otsuka snaps up cancer drugmaker Astex"
E4. Royal Society Tom Blundell Biography
E5. Astex webpage details of the five drugs undergoing clinical trials
E6. Business Wire – 31-8-2020 "Astex Pharmaceuticals Announces That Tolinapant (ASTX660)... Has Been Granted Orphan Drug Designation for the Treatment of T-cell Lymphomas by the US FDA"
E7. Combined documents detailing drug approvals including: FDA and EMA approval for Kisqali, Genetic Engineering & Biotechnology News on the FDA approval of Kisqali, FDA approval of Balversa, FDA approval of Inqovi and Astex press release on Inqovi approval.

Information cited above can be found on the following pages: Kisqali approved in 75 countries pg 9; Kisqali FDA and EMA approvals pgs 1 and 2; Balversa breakthrough designation pg 12 and 13; Balversa is the first personalised treatment pg 12.

E8. Novartis – Kisqali Sales 2019 "Novartis delivered strong sales growth, margin expansion and breakthrough innovation launching five NMEs in 2019". Annual sales data on page 5.

E9. Novartis – Kisqali 01.06.2019 "Novartis Kisqali significantly extends life in women with HR+/HER2- advanced breast cancer in MONALEESA-7 trial"

E10. Breast Cancer Now article describing benefits of Kisqali

E11. BusinessWeekly – Astex and Jenssen partnership for Balversa 16.03.2018 "Astex IP targeted at potential $1 billion market"