Institution: The University of Manchester

Unit of Assessment: 1

Title of case study:
Diagnostics and novel life-saving therapies for aspergillosis

1. Summary of the impact

Research at the University of Manchester (UoM) has changed the landscape of medical care and research in fungal infections internationally. The impacts include: the world's first commercialised molecular diagnostic products for aspergillosis and *Pneumocystis* pneumonia (£10m investment); pivotal contributions to the preclinical development (£35m investment), clinical developments and registrations of 3 new antifungals with combined market share of ~$2 billion; one (voriconazole, 2012 sales >$750m worldwide) now first line therapy for invasive aspergillosis with improved survival of 15-20%; and internationally validated methods to detect azole resistance in *Aspergillus* (an emerging problem partly related to environmental spraying of azole fungicides for crop protection).

2. Underpinning research

See section 3 for references 1-6. UoM researchers are given in bold.

Key UoM researchers:

- **David Denning** (Senior Lecturer, 1990-1993; Professor, 1993-date)
- **Michael J Anderson** (Research Associate, 1993-1998; Research Fellow, 1998-2006; Scientific Project Manager, 2011-date)
- **Caroline Moore** (Honorary Research Associate, 2008-date)
- **Peter Warn** (Research Associate, 1997-2005; Senior Scientist, 2005-2007; Senior Lecturer, 2007-date)
- **Paul Bowyer** (Senior Lecturer, 2005-date)
- **Mike Bromley** (Research Fellow, 2008-2013; Lecturer, 2013-date)
- **Nicola Smith** (Research Associate, 2011-date)
- **Susan Howard** (Research Associate, 2008-2012)
- **Robert Niven** (Senior Clinical Research Fellow, 1997-2002; Senior Lecturer, 2002-date)

Clinical and laboratory research led by **Denning** has resulted in the following discoveries:

- Means of detecting azole resistance in A. fumigatus (**Moore**), validated in animal models (**Warn**), and two mechanisms of resistance (target site mutation and efflux). Azole resistance methodology development was followed up by documentation of the clinical impact of resistance in Manchester (**Denning**) (1) and lately by detection of resistance using molecular methods (**Bowyer, Smith, Denning**), in the absence of a positive culture, never before done for a human pathogenic fungus (2).

- Genome sequencing of A. fumigatus strain AF293 (29,000 Mb, ~9,700 genes) was published in 2005 (3). **Denning** led the genome sequencing of A. fumigatus with seed funding from the Wellcome Trust, followed up by a further Wellcome Trust grant (to Sanger) and a $3m NIH grant (to UoM, partly subcontracted to The Institute for Genomic Research, now J Craig Venter Institute, Rockville, MD). The strain sequenced derived from **Denning**'s clinical collection. In parallel A. nidulans and A. oryzae were sequenced elsewhere with **Denning** co-ordinating the analysis and publication outputs in multiple meetings until publication. All subsequent genomic studies of filamentous studies use AF293 as the reference Aspergillus strain.

- **Denning** led the development of the antifungal drug voriconazole and made a major contribution to the development of caspofungin and micafungin. The first patients in the world were treated in Manchester with voriconazole in 1993. **Denning** analysed the clinical and radiological outcomes for the phase 2 aspergillosis studies (published 2002). He led the protocol development for the randomised registration studies, facilitating co-working of Pfizer

and the European Organisation for the Research and Treatment of Cancer (EORTC), designing data collection processes and training for the data analysis teams (one US and one European). He led the European data analysis team over 3 years. **Denning** wrote the first protocol draft in 1997 and the combined studies were successfully completed in 2001 and published in 2002 (4). Denning was one of 3 adjudicators of eligibility and outcome of invasive aspergillosis cases recruited into the caspofungin study and international studies of micafungin, a novel class of echinocandin antifungal drug.

- Denning and colleagues have redefined certain clinical manifestations of aspergillosis using clinical observation and both old and new diagnostic tools. Chronic cavitary pulmonary aspergillosis was introduced in 2003 (5) and antifungal benefit documented for the first time. Azole resistance is a particular problem in this group, because of long term antifungal therapy (1) (**Howard**). The link between severe asthma and fungal sensitisation (SAFS), a term coined by **Denning**, **Niven** and colleagues, and its responsiveness to oral antifungal therapy was exemplified in a double blind, placebo-controlled RCT published in 2009 (6). For responsive patients, the quality of life impact is as large as prednisolone and larger than Omalizumab.

3. References to the research

Azole resistance

1. **Howard SJ**, Cesar D, **Anderson MJ**, Albarrag AM, Fisher M, Pasqualotto AC, Laverdiere M, Arendrup MC, Perlin DS, **Denning DW**. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerging Infectious Diseases*. 2009;15:1068-76. DOI: 10.3201/eid1507.090043

2. **Denning DW**, Park S, Lass-Florl C, Fraczek MG, Kirwan M, Gore R, Smith J, Bueid A, **Bowyer P**, Perlin DS. High frequency triazole resistance found in non-culturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clinical Infectious Diseases*. 2011;52:1123-9. DOI: 10.1093/cid/cir179

Genome sequencing

3. Nierman W, Pain A, **Anderson MJ**, Wortman J, Kim HS, Arroya J, Berriman B, Abe K, Archer DB, Bermejo C, Bennett J, **Bowyer P**, Chen D, Collins M, Coulsen R, Davies R, Dyer PS, Farman M, Federova N, Feldblyum TV, Fisher R, Fosker N, Fraser A, García JL**,** García MJ, Goble A, Goldman GH, Gomi K, Griffith-Jones S, Gwilliam R, Haas B, Harris D, Horiuchi H, Huang J, Humphrey S, Jiménez J, Keller N, Khouri H, Kitamoto K, Kobayashi T, Konzack S, Kulkarni R, Kumagai T, Lafton A, Latgé JP, Lord A, Lu C, Majoros WH, May GS, Miller BL, Mohamoud Y, Molina M, Monod M, Mouyna I, Mulligan S, Murphy L, O'Neil S, Paulsen I, Penalva MA, Pertea M, Price C, Pritchard BL, Quail MA, Rabbinowitsch E, Rawlins N, Rajandream M-A, Reichard U, Renauld H, Robson GD, de Córdoba SR, Rodríguez-Peña JM, Ronning CM, Rutter S, Salzberg SL, Sanchez S, Sánchez-Ferrero JC, Saunders D, Seeger K, Squares R, Squares S, Takeuchi T, Tekaia F, Turner G, Vazquez de Aldana CR, Weidman J, White O, Woodward J, Yu J-H, Fraser C, Galagan JE, Asai K, Machida M, Hall N, Barrell B, **Denning DW**. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus. Nature*. 2005;438:1151-6. DOI: 10.1038/nature04332

(Two related papers in same issue of *Nature* reporting sequencing of *A. nidulans* and *A. oryzae*.)

Antifungal drug development

4. Herbrecht R, **Denning DW**, Patterson TF, Bennett JE, Greene RE, Oestmann J-W, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hodges MR, Schlamm HT, Troke PF, de Pauw B. Voriconazole versus Amphotericin B for Primary Therapy of Invasive Aspergillosis. *The New England Journal of Medicine*. 2002;347(6):408-15. DOI: 10.1056/NEJMoa020191
(Several other papers reporting clinical results of antifungal trials published.)

Chronic pulmonary aspergillosis and Severe Asthma with Fungal Sensitisation (SAFS)

5. **Denning DW**, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavitary and fibrosing

pulmonary and pleural aspergillosis: Case series, proposed nomenclature and review. *Clinical Infectious Diseases*. 2003;37 (Suppl 3):S265-80. DOI: 10.1086/376526

6. **Denning DW**, O'Driscoll BR, Powell G, Chew F, Atherton G, Vyas A, Miles J, Morris J, **Niven RM**. Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitisation (SAFS), the FAST study. *American Journal of Respiratory and Critical Care Medicine*. 2009;179:11-8. DOI: 10.1164/rccm.200805-737OC

4. Details of the impact *See section 5 for corroborating sources S1-S10.*

Reach and significance of the impact

Clinical development of voriconazole and echinocandins: saving lives from invasive aspergillosis

The development and subsequent worldwide registration of voriconazole as the most effective first line therapy for invasive aspergillosis has been a critically important development with a 13% absolute survival benefit. All international guidelines now place voriconazole as first line therapy (S1) and numerous post-registration studies show ~15-20% better survival with voriconazole therapy compared with all other therapies. Likewise introduction of the very low toxicity echinocandin antifungals has been pivotal in improved survival with minimal adverse events from life-threatening fungal infections. In 2011, the global antifungals market was ~ $10.7bn, with an annual growth rate of 2.9% during 2002 and 2010. Voriconazole sales were >$750m worldwide in 2012 (S2, p. 12). Caspofungin and micafungin echinocandin sales are over $500m each per year. **Denning** profiled the echinocandins as a new drug class in *The Lancet* in 2003 (S3).

Resistance in Aspergillus

Without validated resistance detection in *Aspergillus* (S4), we would not know that clinical failures are due to resistance (as opposed to inactive drug, immunological failure etc.) or that azole fungicide use in agriculture is leading to an increasing problem (since 2003) in environmental resistance in Aspergillus (European Centres for Disease Control report, 2013) (S5).

Validated susceptibility testing and genome sequencing as a springboard for antifungal drug discovery: F2G Ltd

Validated susceptibility testing of *Aspergillus* provides an antifungal drug discovery tool. This methodology, combined with UoM animal modelling know-how, patented gene knockout techniques and clinical profiling, was used as the foundation of F2G Ltd, a UoM spin out (S6). Genome sequencing of *Aspergillus* greatly accelerated the finding of novel antifungal targets for F2G (and other antifungal discovery units). A total of ~£35m from venture capital funds has been invested in F2G since 2001, employing up to 22 people. F2G's lead compound (F3 analogue) has a novel mode of action and chemical structure and is therefore a new antifungal class, primarily with anti-*Aspergillus* activity. It has no discernable toxicity in small animals. It is formulated for intravenous and oral usage. Phase 1 is anticipated in Q4 2013.

The world's first commercialised molecular diagnostics for *Aspergillus* (respiratory) and *Pneumocystis*

The major limitation to better clinical outcomes of invasive fungal infections remains insensitive and slow diagnosis. To address this issue, **Denning** founded Myconostica to develop and commercialise real-time PCR diagnostics for fungal disease. The MycAssay® Aspergillus and MycAssay® Pneumocystis assays are the world's first commercial real-time quantitative PCR assays for pulmonary fungal infections and both were developed with £10m external funding (S7). Quantitation was possible in *Aspergillus* because the number of ribosomal RNA copies in the *A. fumigatus* genome strain was precisely determined in the sequencing project. CE marking throughout Europe and Canada was achieved after the international clinical trials programme that **Denning** managed. Sales have been made to 16 countries and licensing deals with Becton Dickinson (S8) and BioRad will result in OEM developments on new platforms such as the new fully automated BD MAX™ system, a coming revolution in microbiology.

Redefining chronic pulmonary aspergillosis (CPA) and its global impact

The National Aspergillosis Centre at the University Hospital of South Manchester (S9) was the first

nationally commissioned infectious disease service in the UK and the world's first national clinical centre for a fungal disease. The basis of national commissioning was **Denning**'s clinical expertise, the small national caseload of CPA (<1,000 UK cases), clinical care complexity, need for specialised investigations and antifungal drug cost. In 2013, the centre has about 270 CPA (and over 500 allergic aspergillosis) cases under its care with >250 new referrals annually.

CPA is commonly preceded by pulmonary tuberculosis and an estimate of the global prevalence of cases (assuming ~15% annual mortality) is 1.2m, most in countries without access to any diagnostics for fungal disease and unaffordable antifungal therapy. This is being addressed with the WHO STOP TB programme. The National Aspergillosis Centre designation directly facilitated this global health development, with multiple burden of disease estimates emerging (S10).

Role of fungal allergy in asthma

The placebo-controlled RCT of antifungal therapy in those with SAFS has been pivotal in altering thinking about the pathogenesis of severe asthma. Major quality of life benefits for 60-80% of such patients were found. The global burden of SAFS is estimated at 6-13m adults, with ~100,000 deaths annually, but needs further epidemiological study. Very large numbers of patients are benefitting from generic antifungal therapy for severe asthma, including some children.

5. Sources to corroborate the impact

S1. Walsh TJ, Anaissie EJ, **Denning DW**, et al. Treatment of Aspergillosis: Clinical Practice Guidelines of the Infectious Diseases Society of America (IDSA). *Clinical Infectious Diseases.* 2008; 46: 327-60.

S2. Zacks *Brokerage Research Digest*: Pfizer, Inc., 1Q13 Results (p. 12).

S3. **Denning DW**. Echinocandin antifungal drugs. *The Lancet*. 2003;362:1142-51.

S4. European Committee on Antimicrobial Susceptibility Testing. Rationale documents for antifungal agents: http://www.eucast.org/antifungal_susceptibility_testing_afst/rationale_documents_for_antifungals/

S5. European Centre for Disease Prevention and Control. Risk Assessment on the Impact of Environmental Usage of Triazoles on the Development and Spread of Resistance to Medical Triazoles in *Aspergillus* Species. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/risk-assessment-impact-environmental-usage-of-triazoles-on-Aspergillus-spp-resistance-to-medical-triazoles.pdf

S6. F2G Ltd Completes $30 Million Financing Round to Fund Pre-clinical and Clinical Development of Novel Anti-fungal Compounds: http://www.f2g.com/05_Sep_2012.htm

S7. www.myconostica.co.uk/latest-news/y2011

S8. BD and Lab21 Collaborate to Develop *Aspergillus* Assay for New BD MAX™ Molecular Testing System: www.bd.com/contentmanager/b_article.asp?Item_ID=26368&ContentType_ID=1&BusinessCode=20001&d=BD+Worldwide&s=&dTitle=&dc=&dcTitle=

S9. National Aspergillosis Centre: www.nationalaspergillosiscentre.org.uk

S10. Multi-country burden of fungal disease presented at ECCMID conference, 2013: http://www.life-worldwide.org/media-centre/article/multi-country-burden-of-fungal-disease-presented-at-eccmid-conference/