PROJECT FINAL REPORT

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Executive Summary

The development of novel technologies to diagnose and treat *Aspergillus fumigatus* infections in humans was the focus of the MATHIAS research consortium. *Aspergillus fumigatus* is a ubiquitous environmental mould whose air-borne spores are inhaled into the lungs. While healthy individuals with functioning immune systems are rarely infected by the fungus, immuno-compromised individuals, particularly neutropenic patients with haematological malignancies and bone marrow transplant recipients, are especially susceptible to invasive lung infections which rapidly disseminate throughout the body. Invasive lung infection caused by *A. fumigatus* is known as invasive pulmonary aspergillosis (IPA), which carries an unacceptably high rate of mortality, typically approaching 40%. Patient prognosis worsens significantly in the absence of an accurate diagnosis and timely and appropriate treatment. At present, there is no single 'gold standard' test for IPA, with detection relying on a number of diagnostic indicators that lack either specificity or sensitivity, which suffer from lengthy turnaround times, or which rely on invasive biopsy that is not possible in severely sick patients. Consequently, proven diagnosis of the disease is only truly obtained at autopsy. The current lack of accurate and rapid diagnostic tests for IPA is leading to the unnecessary use of costly and toxic antifungal drugs, and is contributing to the emergence of antifungal drug resistance in clinical strains of *A. fumigatus*. Specific, sensitive, and non-invasive detection of the early stages of *A. fumigatus* lung infections would have considerable positive impacts on patient survival and morbidities, and would be of substantial financial benefit to healthcare systems, reducing the current estimated cost of €400,000 due to prolonged hospital stay and antifungal drug expenditure. In addition, real-time monitoring of IPA in response to treatment would enable the dose and duration of therapy tailored to the needs of the patient. Non-invasive diagnosis of IPA, based on state-of-the-art molecular imaging, could provide a rapid and virtually stress-free means of detecting *A. fumigatus* lung infections *in vivo*, and for tracking responses to antifungal treatment.

The approach of the MATHIAS consortium was to develop novel disease-specific tracers for IPA by combining a humanised version of the *Aspergillus*-specific monoclonal antibody JF5 with cutting-edge nuclear medicine technologies incorporating Positron Emission Tomography (PET) and Magnetic Resonance Imaging (PET/MRI). Our newly developed antibody-guided PET tracer [^{64}Cu]-NODAGA-hJF5 shows excellent results in pre-clinical trials, demonstrating clear diagnosis of IPA in an animal model of infection. Details of these ground-breaking studies have recently been published in a number of international high-ranking journals (e.g. Davies G, et al., Theranostics, 2017; 7 (14): 3398-3414; Rolle AM, et al., Proc Natl Acad Sci USA, 2016; 113 (8): E1026-33). Translation of the technology to the clinical setting and use of the tracer in humans requires production of its component parts (humanised JF5 antibody and NODAGA chelator) under stringent GMP conditions. Due to budget limitations we were only able to have small amounts of GMP-grade hJF5 and GMP-grade p-NCS-benzyl-NODAGA chelator manufactured for us by licensed European sub-contractors. Despite this, we successfully coupled the GMP chelator and GMP antibody according to GMP guidelines, and conducted rigorous safety tests which showed no adverse effects of the tracer in toxicology studies. Further work is needed to fully validate the conjugation procedures and to undertake Phase I / II clinical trials in humans. Notwithstanding this, we have developed and safety-tested sufficient GMP-grade tracer to allow a first-in-human trial on compassionate grounds. This trial is currently underway in Germany, while we seek funding and approval for larger-scale trials.

Summary description of project context and objectives

The development of novel technologies to diagnose and treat lung infections caused by the opportunistic fungal pathogen *Aspergillus fumigatus* was the aim of the European research consortium MATHIAS. Invasive pulmonary aspergillosis (IPA) is a life-threatening lung disease of immunocompromised patients (typically neutropenic haematological malignancy and bone marrow transplant patients) caused by the ubiquitous environmental mould *A. fumigatus*. Diagnosis of this disease is extremely difficult since radiological signs in computed tomography scans are not specific for the disease, and so culture of the fungus from lung biopsies is needed for accurate diagnosis. Biopsy is an invasive procedure that is often not possible in severely sick patients, and so the MATHIAS consortium has used an *Aspergillus*-specific specific monoclonal antibody (JF5) developed by ISCA Diagnostics, in an antibody-guided imaging procedure known as immuno-PET/MR, which allows non-invasive detection of *Aspergillus* lung infections *in vivo*. This 5-year project had four work packages (management included).

The objective of WP1, led by ISCA, was to generate antibody fragments (scFv, Fab and $F(ab')_2$) and a humanised version of JF5 (hJF5). Once generated, the fragments and full-length mJF5 and hJF5 retained functional immuno-reactivity with the target antigen. The proteins were then conjugated with a metal chelator for radionuclide ^{64}Cu labelling and analysed at PSI. This allowed pre-clinical imaging of IPA in a mouse model of disease.

WP2, led by EKUT and supported by UKEssen, focused on the pre-clinical evaluation of the candidate immuno-conjugates *in vitro*, *in vivo* and *ex vivo*, and their ability to detect *A. fumigatus* with a high degree of specificity and sensitivity. Using fluorochrome-labelled antibodies and their fragments, *A. fumigatus* lung infection was investigated using confocal and light-sheet microscopy in neutropenic mice, providing real-time high resolution imaging of disease progression. Concurrently, [^{64}Cu]-labelled antibodies and immuno-PET/MR imaging were used to track IPA in the neutropenic mouse model, with close concordance of results with high-resolution microscopy. With an emphasis on specificity, this work package provided the requisite pre-clinical data for diagnostic efficacy needed for translation of the tracer into the clinical setting. Finally, the potential of the newly developed radio-immunoconjugate for monitoring IPA responsiveness to antifungal drugs was investigated.

The goal of WP3 was translation of the most suitable radio-immunoconjugate from bench-to-bedside. This required production of the component parts of the radio-immunoconjugate (chelator, antibody and radionuclide) in accordance with Good Manufacturing Practice (GMP) procedures to ensure quality, reproducibility and suitability for human use. The establishment of the conjugation protocol was supported by PSI. In parallel, the radio-immunoconjugate needed to be fully validated for stability and reactivity and, critically, toxicity. Lastly, in anticipation of a human clinical study, documentation for registration and approval of a trial by institutional ethics committees (IEC), and by the Paul Ehrlich Institute (PEI) as the regulatory authority, was prepared. However, due to time and budget restrictions, a human clinical study was not possible during the course of the MATHIAS project.

Description of main S&T results/foregrounds

In WP1, we successfully generated fragments of the *Aspergillus*-specific mouse monoclonal antibody JF5 (mJF5) by expression of recombinant scFv and Fab in the bacterium *Eschericia coli*, and have successfully developed a stable cGMP-grade Chinese Hamster Ovary (CHO) cell line expressing humanised JF5 antibody (hJF5) following grafting of the mouse JF5 complementarity

determining regions (CDRs) into a human IgG1 framework. In addition to recombinant scFv and Fab fragments, we have generated Fab and $F(ab')_2$ fragments of mJF5 and hJF5 enzymatically using papain and pepsin digestion of the full-length antibodies. The antibody fragments and full-length humanised antibody retain their specificity to *Aspergillus*, binding to active (invasive) growth phases of the pathogen only. Using the full-length mJF5 and hJF5 antibodies, we have explored a number of chelators and chemistries for conjugating the radionuclide [^{64}Cu] to the antibodies, and work in WP2 has shown that JF5, in combination with the chelator NODAGA, allows highly specific detection of IPA *in vivo*, with minimal uptake of the tracer by non-target organs such as the liver, and no accumulation of the antibody-based tracer in lungs infected by other pathogens. We have also shown that the disease-specific tracer [^{64}Cu]NODAGA-hJF5 has superior PET/MR imaging capabilities compared to its mouse counterpart. Importantly, we have characterised the antigenic determinant bound by JF5 as β1,5-galactofuranose (Gal*f*), an *Aspergillus*-specific epitope not found in mammalian carbohydrates. This dramatically reduces the likelihood of adverse effects of the tracer in humans due to non-specific binding to host tissues. The outputs of WP1 and WP2 have demonstrated the high specificity and sensitivity of the tracer for real-time *in vivo* imaging of *Aspergillus* lung infections, providing a highly accurate and sensitive means of diagnosing early stages of infection and monitoring disease responsiveness to antifungal treatment. Altogether, our newly developed and highly novel antibody-based tracer [^{64}Cu]NODAGA-hJF5 has proven to be an excellent candidate for clinical diagnosis of IPA in humans. Throughout the work, we have gained new insights into the pathobiology of IPA by harnessing the unique capabilities of immunoPET/MR and confocal and light sheet imaging.

In fulfilment of WP3, a specialist company was sub-contracted to generate a Master Cell Bank of the hJF5 CHO cell line, and to produce humanised JF5 monoclonal antibody (hJF5) under cGMP conditions. *In vitro* assays have shown long-term stability and sustained immuno-reactivity of the GMP-grade hJF5 antibody when maintained under standardised storage conditions. Refinements to the production of the chelator (R)-NODAGA$(tBu)_3$ over the course of the project has resulted in a chemical purity of >99.5%, while optimisation of the production and purification protocols for [^{64}Cu] has enabled GMP-grade radionuclide production. Toxicity tests with the NODAGA-hJF5 conjugate, produced according to GMP guidelines, were performed by a licensed sub-contractor, and no adverse effects of the hJF5 antibody were demonstrated. Finally, a study protocol for a small-scale first-in-human clinical study has been developed, although the study itself will be conducted outside of the MATHIAS project.

Potential impact and main dissemination activities and exploitation results

Potential impact

The antibody-guided tracer developed by the MATHIAS consortium is expected to make a positive contribution to the management of patients at high-risk for *Aspergillus* infections (neutropenic haematological malignancy patients, bone marrow and solid organ transplant recipients, individuals with underlying respiratory diseases, and those with AIDS) providing rapid, sensitive and highly-specific detection of *Aspergillus* infections without the need for invasive biopsy. The disease-specific tracer will have a positive impact on survival rates, reducing morbidities, and helping limit the unnecessary exposure of patients to costly and toxic antifungal drugs, by providing diagnostic-driven treatment rather than prophylactic or empirical therapy.

Our work is at the forefront of molecular imaging for infectious diseases, especially invasive fungal disease (IFD), having developed and applied this technology not only to the detection of IPA but also to invasive candidiasis, a blood-borne IFD caused by the yeast *Candida albicans*. Antibody-guided molecular imaging for diagnosis and therapy monitoring of IFDs is currently not used in

clinical practice. Rather, non-specific enrichment of metabolic tracers, such as radioactively labelled glucose ([^{18}F]FDG), is currently being pursued. While this tracer is well suited to cancer diagnostics, we have shown in our work that it is unsuitable for infectious disease detection and the differentiation of IPA from other infectious etiologies in the lung. Our work has pioneered the early and specific non-invasive diagnosis of IPA by harnessing the supreme specificity of a monoclonal antibody with the sensitivity of PET/MRI. This previously unexplored approach to IFD diagnosis is highly innovative, and our pre-clinical studies have demonstrated the enormous potential of immunoPET/MRI to detect *Aspergillus* lung infections *in vivo*. Successful humanisation of the *Aspergillus*-specific monoclonal antibody JF5 and characterisation of its target epitope has made translation of the humanised tracer to the clinical setting a reality, culminating in a small-scale first-in-human study (albeit beyond the timeline of the MATHIAS project).

Our work goes beyond IPA diagnostics however. Leading manufacturers of state-of-the-art imaging hardware used in this project are based in Europe. Combining this market lead in imaging hardware with the advanced molecular imaging approaches developed by us has enhanced the quality and competitiveness of European research and innovation in molecular imaging of infectious diseases. Research and development of antibody-based approaches to the detection and treatment of infectious diseases is a rewarding field for innovative SMEs in Europe, and has established our project SME partners as industry leads in the development and supply of high integrity antibodies and diagnostic tests for human pathogenic fungi (ISCA), and production of high purity chelators (CHEMA).

Dissemination activities

The results of the project have been presented at 20 domestic and international conferences, and through web-based forums and industry blogs. Additionally, the project was shortlisted for the Galenus von Pergamon Prize 2016, and was therefore presented to a wider audience during the gala performance. Furthermore, the results of the project have been published in a number of international peer-reviewed journals:

- H. O. J. Morad, A. M. Wild, S. Wiehr, G. Davies, A. Maurer, B. J. Pichler, C. R. Thornton. (2018). Pre-clinical Imaging of Invasive Candidiasis Using ImmunoPET/MR. *Frontiers in Microbiology* **9:** 1996. DOI:10.3389/fmicb.2018.01996.
- C. R. Thornton. (2018). Molecular Imaging of Invasive Pulmonary Aspergillosis Using ImmunoPET/MRI: The Future Looks Bright. *Frontiers in Microbiology* **9:** 691. DOI:10.3389/fmicb.2018.00691.
- G. W. Severin, L. K. Kristensen, C. H. Nielsen, J. Fonslet, A. I. Jensen, A. F. Frellsen, K. M. Jensen, D. R. Elema, H. Maecke, A. Kjaer, K. Johnston, U. Koster, Neodymium-140 DOTA-LM3: Evaluation of an In Vivo Generator for PET with a Non-Internalizing Vector. (2017). *Frontiers in Medicine* **4:** 98. DOI:10.3389/fmed.2017.00098.
- P. R. Spycher, C. A. Amann, J. E. Wehrmuller, D. R. Hurwitz, O. Kreis, D. Messmer, A. Ritler, A. Kuchler, A. Blanc, M. Behe, P. Walde, R. Schibli. (2017). Dual, Site-Specific Modification of Antibodies by Using Solid-Phase Immobilized Microbial Transglutaminase. *Chembiochem: a European Journal of Chemical Biology* **18:** 1923-1927. DOI:10.1002/cbic.201700188.
- G. Davies, A. M. Rolle, A. Maurer, P. R. Spycher, C. Schillinger, D. Solouk-Saran, M. Hasenberg, J. Weski, J. Fonslet, A. Dubois, F. Boschetti, F. Denat, M. Gunzer, M. Eichner, L. S. Ryder, M. Jensen, R. Schibli, B. J. Pichler, S. Wiehr, C. R. Thornton. (2017). Towards Translational ImmunoPET/MR Imaging of Invasive Pulmonary Aspergillosis: The

Humanised Monoclonal Antibody JF5 Detects Aspergillus Lung Infections *In Vivo*. *Theranostics* **7:** 3398-3414. DOI:10.7150/thno.20919.

- J. Fonslet, S. Tietze, A. I. Jensen, S. A. Graves, G. W. Severin. (2017). Optimized procedures for Manganese-52: Production, Separation and Radiolabeling. *Applied Radiation and Isotopes*: *Including Data, Instrumentation and Methods for use in Agriculture, Industry and Medicine* **121:** 38-43. DOI:10.1016/j.apradiso.2016.11.021.
- S. Wiehr, P. Warnke, A. M. Rolle, M. Schutz, P. Oberhettinger, U. Kohlhofer, L. Quintanilla-Martinez, A. Maurer, C. Thornton, F. Boschetti, G. Reischl, I. B. Autenrieth, B. J. Pichler, S. E. Autenrieth. (2016). New Pathogen-Specific ImmunoPET/MR Tracer for Molecular Imaging of a Systemic Bacterial Infection. *Oncotarget* **7:** 10990-11001. DOI:10.18632/oncotarget.7770.
- A. M. Rolle, M. Hasenberg, C. R. Thornton, D. Solouk-Saran, L. Mann, J. Weski, A. Maurer, E. Fischer, P. R. Spycher, R. Schibli, F. Boschetti, S. Stegemann-Koniszewski, D. Bruder, G. W. Severin, S. E. Autenrieth, S. Krappmann, G. Davies, B. J. Pichler, M. Gunzer, S. Wiehr, ImmunoPET/MR Imaging Allows Specific Detection of *Aspergillus fumigatus* Lung Infection *In Vivo*. (2016). *Proceedings of the National Academy of Sciences of the United States of America* **113:** E1026-1033. DOI:10.1073/pnas.1518836113.
- G. W. Severin, J. T. Jorgensen, S. Wiehr, A. M. Rolle, A. E. Hansen, A. Maurer, M. Hasenberg, B. Pichler, A. Kjaer, A. I. Jensen. (2015). The Impact of Weakly Bound (8)(9)Zr on Preclinical Studies: Non-Specific Accumulation in Solid Tumors and *Aspergillus* Infection. *Nuclear Medicine and Biology* **42:** 360-368. DOI:10.1016/j.nucmedbio.2014.11.005.
- H. F. Wehrl, S. Wiehr, M. R. Divine, S. Gatidis, G. T. Gullberg, F. C. Maier, A. M. Rolle, J. Schwenck, W. M. Thaiss, B. J. Pichler. (2014). Preclinical and Translational PET/MR Imaging. *Journal of Nuclear Medicine* **55:** 11S-18S. DOI:10.2967/jnumed.113.129221.
- C. R. Thornton (2014). Breaking the Mould - Novel Diagnostic and Therapeutic Strategies for Invasive Pulmonary Aspergillosis in the Immune Deficient Patient. *Expert Review of Clinical Immunology* **10:** 771-780. DOI:10.1586/1744666X.2014.904747.

Exploitation of results

The exploitable foreground from the MATHIAS project surrounds the development of the humanised JF5 antibody and its use in immunoPET/MR imaging of invasive pulmonary aspergillosis (IPA) in humans. Protection of the Background IP (the full-length mouse antibody JF5, its CDRs, and functional antibody fragments thereof) is already sought under pending patent applications (WO10082034, US2015018532, US2012064093, CN102439038, EP2387585 and IN05474DN2011). As a consortium, we will use our best efforts to exploit the foreground generated during the MATHIAS project in order to commercialise the antibody tracer for clinical diagnosis of IPA. Commercialisation is first reliant on proof of diagnostic efficacy through an ongoing 'first-in-human' clinical trial. However, we have already taken steps to engage with downstream partners (industry/pharma/biotech). Our highly successful project has been identified through the Horizon 2020-funded UTILE initiative (www.health-breakthrough.eu) as one of a number of FP7-funded projects as having real potential for commercialisation. To this end we are currently seeking licensing and funding opportunities through LifeArc (www.lifearc.org) to enable commercialisation of our ground-breaking work.

In addition, during the MATHIAS project, Dr. Philipp Spycher has filed a patent that aims to protect a new technology that allows site-specific attachment of payloads (e.g. anti-cancer drugs) to native antibodies. This then allows the generation of therapeutic antibody-drug conjugates (ADCs) by attaching highly cytotoxic compounds for cancer therapies, or for diagnostic applications by

attaching a moiety suitable for non-invasive imaging. The ADCs generated with this technology appear to be highly stable and have excellent pharmacokinetic properties, which might result in better therapeutic outcome with lower side-effects. This patent-protected technology was the foundation for establishing a new spin-out company called Araris Biotech AG (www.ararisbiotech.com) during the course of the MATHIAS project.

Finally, the chelator p-NCS-Benzyl-NODA-GA refined during the MATHIAS project will be commercialised by CHEMA. The chelator shows promising properties and is of interest to CHEMA's clients. The cGMP production of this compound will allow its use for clinical trials, and potentially will lead to new commercial products in combination of other biomolecules.

Address of project website and relevant contact details

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