Institution: Nottingham Trent University (NTU)

Unit of Assessment: A03 – Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Improving the Management and Survival of Patients with Aggressive, Treatment-Resistant Cancers

Period when the underpinning research was undertaken: 2016 - present

Details of staff conducting the underpinning research from the submitting unit:

Names:	Roles:	Periods employed by submitting HEI:
Sergio Rutella	Professor of Cancer Immunotherapy	2016-present
Jayakumar Vadakekolathu	Research Fellow	2012-present
Gemma A. Foulds	Research Fellow	2013-present

Period when the claimed impact occurred: 2018 to 30 September 2020

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact

NTU-led research identified immune signposts or "biomarkers" that predict patients with the chemotherapy-refractory form of acute myeloid leukaemia (AML) who might respond to MacroGenics' immunotherapy drug flotetuzumab. NTU's research also informed the development of a paediatric arm of the flotetuzumab clinical trial. NTU's work, hence promoted the positive perception of the drug by investors and biotech equity analysts, and motivated MacroGenics' $19.5M investment in developing the drug. NTU's 'precision medicine' approach, advice and testing of molecular profiling techniques enabled successful spatially resolved analyses of bone marrow biopsies at unprecedented depth using NanoString's GeoMx DSP platform. This development, demonstration and showcasing of the product's capabilities for research in haematological malignancies has been a key factor in NanoString's market capitalization growth from $242 million to $1.14 billion in 5 years.

2. Underpinning research

Leading an international team and leveraging financial support from research charities (**G1**, **G2**) and commercial entities (**G3**, **G4**, **G5**), Rutella within the John van Geest Cancer Research Centre (JvGCRC) set out to characterise the 'immune ecosystem' of AML and to identify immune gene signatures that are reflective of general immune status and predictive of anti-leukaemia immune potential. The research used 3D biology technologies, including targeted immune transcriptomic profiling and spatially resolved digital immunohistochemistry, to characterise the immune ecosystem of the bone marrow tumour microenvironment (TME) in 42 children and 28 adults with AML. This identified heterogeneous immune profiles and concluded that 'immune enriched' AMLs might be amenable to immunotherapy approaches tailored to the bone marrow TME (**R1**).

AML is characterized by clonal expansion of poorly differentiated myeloid precursors, resulting in impaired haematopoiesis and often bone marrow failure. According to Cancer Research UK, an average of 3,200 new cases of AML are diagnosed in the UK annually and 2,600 patients die from AML each year. In the United States, the American Cancer Society estimated that 19,940 people were diagnosed with AML in 2020 and 11,180 people died from the disease. The general therapeutic strategy has not changed substantially in more than 30 years, with chemotherapy remaining the standard of care for most patients with AML despite recent drug approvals by the U.S. Food and Drug Administration. AML is cured in only 35 to 40 per cent of patients below 60 years of age and in 5 to 15 per cent of patients above 60 years of age. While chemotherapy resistance is common, relapse is the most common cause of treatment failure, with only 10% of patients surviving 5 years or longer after disease recurrence.

In the first study of its kind into the genetic make-up of bone marrow cells in leukaemia, the research team used machine learning to identify a gene signature that predicts outcomes for patients with AML. Using an artificial neural network-based machine learning approach to analyse a publicly available dataset for 593 adults with AML, they found that a patient may have a better chance of survival, and may not require intensive treatment, if genes coding for *CALCRL*, *CD109* and *LSP1* are switched off (**R2**). These findings led to the establishment of new sub-categories of risk for which different treatment options should be offered to maximise patient benefit while keeping unwanted toxicity to a minimum, and the early-stage development of a companion diagnostic device to support treatment decision making.

Using innovative RNA/protein expression and digital spatial profiling approaches, researchers led by Rutella carried out high-dimensional analysis of the immunological structure of 442 primary bone marrow samples from patients with AML, unravelling critical differences in immune gene expression across age groups and disease stages. The study also identified those patients that are more likely to fail standard chemotherapy. Crucially, the study found that patients with an interferon (IFN-γ) dominant TME (the 'hot' or 'inflamed' AML immune subtype) are more likely to respond to immunotherapy with flotetuzumab (FLZ), a novel CD123×CD3 dual affinity re-targeting molecule currently in a phase I clinical trial (**R3**, **R4**) sponsored by MacroGenics. This study led to MacroGenics filing three patents, with Rutella and NTU named as a co-inventor, relating to bispecific CD123×CD3 diabodies for the treatment of hematologic malignancies (**R5**).

The AML research programme as a whole has involved the molecular profiling of an unrivalled cohort of AML samples provided by clinical collaborators (>600 specimens). Rutella used this to generate a compendium of AML gene and protein profiles that provide novel insights into the immunobiology of the disease. These are being made available in the Gene Expression Omnibus, an open-access repository of high throughput transcriptomic data (accession numbers: GSE134589 and GSE146204).

This novel methodological approach extends beyond AML and is informing the development of immunotherapy clinical trials for patients with advanced (metastatic) gastric cancer (**R6**).

3. References to the research

The quality of the underpinning research has been evidenced by the following selected rigorously externally peer reviewed outputs and patented inventions, and by their use and exploitation for further research beyond NTU:

R1. **Rutella S**, **Vadakekolathu J**, Patel T, Reeder S, Schmitz M, Schaarschmidt H, Warren SE, Liang Y, Hood T, Danaher P, Cesano A, Beechem JM, Pockley AG, Tasian SK, Bornhäuser M. Capturing the complexity of the immune microenvironment of acute myeloid leukemia with 3D biology technology. *Journal of Clinical Oncology* 2018; 36 (Suppl. 5S; A#50). http://doi.org/10.1200/JCO.2018.36.5_suppl.50.

R2. Wagner S, **Vadakekolathu J**, Tasian SK, Altmann H, Bornhäuser M, Pockley AG, Ball GR, **Rutella S**. A parsimonious 3-gene signature predicts clinical outcomes in an acute myeloid leukemia multicohort study. *Blood Advances* 2019; 3: 1330-46. https://doi.org/10.1182/bloodadvances.2018030726. PMID: 31015209.

R3. **Vadakekolathu J**, Minden MD, Hood T, Church SE, Reeder S, Altmann H, Sullivan AH, Viboch EJ, Patel T, Ibrahimova N, Warren SE, Arruda A, Liang Y, Smith TH, **Foulds GA**, Bailey MD, Gowen-MacDonald J, Muth J, Schmitz M, Cesano A, Pockley AG, Valk PJM, Löwenberg B, Bornhäuser M, Tasian SK, Rettig MP, Davidson-Moncada JK, DiPersio JF, **Rutella S**. Immune landscapes predict chemotherapy resistance and immunotherapy response in acute myeloid leukemia. *Science Translational Medicine* 2020; 12(546): eaaz0463. DOI: https://doi.org/10.1126/scitranslmed.aaz0463. PMID: 32493790.

R4. Uy GL, Aldoss I, Foster MC, Sayre PH, Wieduwilt MJ, Advani AS, Godwin JE, Arellano ML, Sweet K, Emadi A, Ravandi F, Erba HP, Byrne M, Michaelis LC, Topp MS, Vey N, Ciceri F, Carrabba MG, Paolini S, Huls G, Jongen-Lavrencic M, Wermke M, Chevallier P, Gyan E, Recher C, Stiff P, Pettit K, Löwenberg B, Church S, Anderson EK, **Vadakekolathu J**, Santaguida MT, Rettig MP, Muth J, Curtis T, Fehr E, Guo K, Zhao J, Bakkacha O, Jacobs K, Tran K, Kaminker P, Kostova M, Bonvini E, Walter RB, Davidson-Moncada JK, **Rutella S**, DiPersio JF. Flotetuzumab as salvage immunotherapy for refractory acute myeloid leukemia. *Blood* 2020 Sep 14: blood.2020007732. DOI: 10.1182/blood.2020007732. PMID: 32929488.

R5. USA Patents Pharmaceutical Formulations of Bispecific CD123 × CD3 Diabodies for the Treatment of Hematologic Malignancies (Attorney Docket No. 1301.0161P; filed on 30 October 2018 and assigned Serial No. 62/752,659); Bispecific CD123 × CD3 Diabodies for the Treatment of Hematologic Malignancies (Attorney Docket No. 1301.0161P2; filed on 19 November 2018 and assigned Serial No. 62/769,078); and Use of Bispecific CD123 × CD3 Diabodies for the Treatment of Hematologic Malignancies (United States Patent Application Serial No. 63/041,051; filed on 20 June 2020).

R6. **Rutella S**, Church SE, **Vadakekolathu J**, Reeder S, Sullivan A, Warren S, Baughman J, Muth

J, Park H, Uronis H, Kang YK, Ng M, Enzinger P, Lee KW, Huber K, Wynter-Horton A, Li D, Bang YJ, Davidson-Moncada J, Catenacci D. Evaluation of tumor microenvironment identifies immune correlates of response to combination immunotherapy with margetuximab and pembrolizumab in HER2+ gastroesophageal adenocarcinoma. *Annals of Oncology* 2019; 30 (Suppl. 5; 123A). DOI: https://doi.org/10.1093/annonc/mdz239.034.

The high quality of the underpinning research is further indicated by the following funding investments in the research and its dissemination:
G1. National Priority Research Project (NPRP8-2297-3-494), Qatar National Research Fund (£630,500)
G2. Roger Counter Foundation, Dorset, UK (£116,000)
G3. NanoString Technologies, Seattle, WA, USA (£64,300)
G4. MacroGenics, Rockville, MD, USA (£157,584)
G5. Kura Oncology, San Diego, CA, USA (£120,828)

4. Details of the impact

NTU research has shaped the design of immunotherapy clinical trials for the treatment, and benefit, of adult patients with aggressive AML. Primary refractory AML, a subtype of the disease, is extremely challenging to treat; the current median survival rate is just four months. To date, no specific treatments have been identified for this patient subset and there is a clear, unmet medical need. Working with MacroGenics, a Nasdaq-listed biopharmaceutical company with annual revenues averaging around $100 million, NTU has carried out an international, multi-centre phase I/II clinical trial (NCT02152956) to determine the maximum tolerated dose level of an immunotherapy drug flotetuzumab in patients with AML, whose disease is not expected to benefit from cytotoxic chemotherapy. The trial has also studied how the drug acts in the body and evaluated its potential anti-tumour activity. MacroGenics' Senior Director of Clinical and Translational Sciences (**S1**) said: "*In my capacity as clinical lead of flotetuzumab development, I can confirm that Sergio's work has been directly applied to our clinical development pipeline. Specifically, Sergio's research has led to modifications in patient selection in clinical trial #NCT02152956 enabling us to identify and include individuals with the 'hot' or inflamed AML immune subtype who are the most likely (45.5%) to benefit from immunotherapy drug flotetuzumab*." More specifically, he said: "*These individuals, who have an immune-infiltrated tumor microenvironment, have chemotherapy-refractory AML (primary induction failure, PIF). Hence, the further benefit of identifying and including these individuals in the trial is that they will avoid overtreatment from cytotoxic chemotherapy that is debilitating and from which they are not likely to derive benefit and be shifted instead to immune based therapies with likely improved responses.*"

Reporting its 2018 end-of-year results to investors, MacroGenics highlighted the presentation of clinical trial data and Rutella's gene signature data at the 2018 American Society of Hematology (ASH) Annual Meeting. Its news release in February 2019 (**S2**) said: "*In the study, flotetuzumab demonstrated anti-leukemic activity and acceptable tolerability in patients with relapsed/refractory AML, with a higher response rate observed in primary refractory patients, an extremely challenging population to treat*." Historically, patients who do not respond to chemotherapy have remission rates to subsequent interventions in the range of only 5-12%, with a median overall survival of 3.5 months. As shown by the updated clinical data presented at the 2020 ASH Annual Meeting (**S3**), "*a remission rate of 32% with good duration and a manageable safety profile observed in the ongoing registrational study of flotetuzumab is very encouraging*". MacroGenics plans to define a potential registration path for flotetuzumab in patients with primary induction failure and early relapsed AML. In August 2019 MacroGenics submitted a briefing document to the US FDA to seek approval for flotetuzumab under the Biologics Licensure Pathway and to seek guidance on other registrational components. The company's Senior Director of Clinical and Translational Sciences confirmed the impact of Rutella's work (**S1**, **S4**): "*Professor Rutella's seminal work has guided and informed our interactions with health regulatory agencies, the Federal Drug Agency of the United States of America. We are currently planning a single-arm, registration-enabling clinical study to evaluate flotetuzumab in up to 200 patients with primary induction failure or early relapse AML. The study will be conducted as a continuation of the ongoing phase I/II study NCT02152956. This is key as it shows a recognition by regulatory agencies of an immune-based classification of AML".*

Rutella's group has also shown that flotetuzumab modulates the immune TME of individuals who are sensitive to its therapeutic effects by inducing the upregulation of PD-L1, a negative immune checkpoint. This observation has impacted on the development of the next generation of combination immunotherapy clinical trials sponsored by MacroGenics by providing a sound rationale for the administration of flotetuzumab in combination with PD-1-blocking antibodies (**S5**). The company confirmed (**S1**): "*Sergio's research identified upregulation of checkpoint inhibitory molecules in a selection of AML patients; this finding helped inform a study of combinatorial immunotherapy in AML, i.e., a new immunotherapy clinical trial that combines flotetuzumab with MGA012, a proprietary anti-PD-1 antibody (clinical protocol #CP-MGD006-03). First patient was dosed on 28 October 2019*." This also supports MacroGenics' collaboration with US pharmaceutical company Incyte; a licensing agreement announced in October 2017, expected to be worth up to $900 million for MacroGenics, gave Incyte exclusive rights to develop and commercialise MGA012, while allowing MacroGenics to develop its own pipeline candidates (in this case flotetuzumab) in combination with the anti-PD-1 checkpoint inhibitor (**S6**).

Development expense for flotetuzumab in 2019 was $15.1 million, which was approximately 8% of total annual research and development expense for MacroGenics, and $4.4 million in the quarter ended March 31, 2020, an increase of 26% compared to the first quarter of 2019 (**S7**). MacroGenics' VP of Investor Relations & Corporate Communications says: "*Professor Rutella's work has contributed positively to the perception of flotetuzumab by investors and biotech equity analysts who follow MacroGenics* […]. *Professor Rutella's oral presentations at major scientific conferences, the recent high-impact scientific publication, and his participation in a key webcast event hosted by MacroGenics for analysts and investors during the American Society of Hematology Meeting in December 2019, have all contributed to a heightened profile for flotetuzumab, as evidenced by inclusion in a number of analysts' proprietary financial models for the Company*".

NTU research has informed the correlative biology studies for the first in-child trial of flotetuzumab in patients with relapsed/refractory AML

The first-in-child PEPN1812 phase 1 trial studying flotetuzumab in pediatric patients with relapsed/refractory AML opened in January 2020 (NCT04158739, **S8**). This is sponsored by the US Children's Oncology Group under the auspices of the US Pediatric Early Phase-Clinical Trial Network. According to the clinical trial description, "*Giving flotetuzumab may stop the leukemia from growing or shrink for a period of time, as well as possibly lessening symptoms, such as pain, that are caused by the leukemia*" (**S8**). The vice-chair of the trial at the Centre for Childhood Cancer Research at the Children's Hospital of Philadelphia wrote, *"PEPN1812 correlative biology studies have benefitted deeply from Sergio's expertise and demonstrated track record of success in the analogous adult patient studies, including his expertise from the adult phase 1/2 flotetuzumab trial (NCT02152956), and Sergio's impactful discoveries of novel immune signature predictors"* (**S9**).

NTU's protocols have delivered commercial benefits to NanoString Technologies through the expansion of their product portfolio for molecular profiling of haematological tumours

NanoString (Seattle, more than 500 employees), develops and commercialises tools for biological research. Its GeoMx Digital Spatial Profiler (DSP) generates whole tissue images at single cell resolution and digital profiling data for DNA, RNA and proteins, enabling researchers to rapidly assess the biological implications of the heterogeneity within tissue samples. NanoString's Chief Scientific Officer (**S10**) confirms that Rutella's research has enabled the company to integrate bone marrow profiling into the platform. He said: *"Dr. Rutella has served as an alpha tester and trusted advisor in the development of a number of our products, including our GeoMx platform, on which he was among the first users to apply the technology to characterizing bone marrow biopsies, a clinically meaningful yet notoriously difficult sample type to work with. Through the expert guidance of Dr. Rutella, NanoString was able to develop protocols that allow us to successfully profile these samples and enable fundamental research in both hematological malignancies and bone metastases of solid tumors."* He also said: *"Dr. Rutella has given multiple seminars and webinars at national meetings and company-sponsored scientific events describing his work and how our technologies enabled it, and these events draw an average attendance of over 300 people. These new contacts are materially important for the company because they represent new users to the platform who will help grow and expand our customer base. […] Dr.*

Rutella's impact on NanoString extends far beyond the field of AML. As a key early and enthusiastic adopter of nCounter and GeoMx, he has given investigators across a wide range of disciplines the confidence to incorporate NanoString technologies into their work".

Rutella's work with NanoString's GeoMx DSP platform has benefitted NanoString's marketing efforts for a platform that costs around £250,000. NanoString's Chief Scientific Officer also said (**S10**): *"In the 5 years that we have been working together, the NanoString market capitalization has grown from $242 million to $1.14 billion. I can't say that it's all due to the work of Dr. Rutella, but without doubt a big element of our success is our ability to do great science with great collaborations such as him*.

5. Sources to corroborate the impact (* participant in the process of impact delivery)

S1.* Testimonial letter and relevant supporting information: Senior Director of Clinical and Translational Sciences, MacroGenics, Inc. (verifies the impact of Rutella's work on the stratification, inclusion and monitoring of patients entering clinical trials).

S2. Web-link: MacroGenics Inc: http://ir.macrogenics.com/news-releases/news-release-details/macrogenics-provides-update-corporate-progress-and-2018;

S3. Web-link: MacroGenics Inc: http://ir.macrogenics.com/news-releases/news-release-details/macrogenics-presents-flotetuzumab-data-patients-refractory-acute;

S4. Web-link: MacroGenics Inc: http://ir.macrogenics.com/news-releases/news-release-details/macrogenics-announces-publication-science-translational-medicine

S5. Web-link: MacroGenics Inc: http://ir.macrogenics.com/news-releases/news-release-details/phase-1-expansion-cohort-oral-presentations-flotetuzumab;

S6.Web-link: MacroGenics Inc: https://www.genengnews.com/topics/drug-discovery/incyte-to-develop-macrogenics-cancer-immunotherapy-mga012-in-up-to-900m-collab/

S7.* Testimonial letter: MacroGenics, Inc. (verifies the impact of Rutella's work on investors and biotech equity analysts).

S8. Web-link: NIH National Library of Medicine, https://clinicaltrials.gov/ct2/show/NCT04158739

S9. Testimonial letter and press release: Children's Hospital of Philadelphia, Philadelphia, USA; https://www.chop.edu/news/study-involving-chop-researchers-could-lead-more-precise-treatment-acute-myeloid-leukemia (verifies Rutella's work on stratification, inclusion, monitoring of paediatric patients entering the first-in-child immunotherapy clinical trial with flotetuzumab).

S10. Testimonial letter: NanoString Technologies, Inc. (verifies Rutella's work on their strategic direction/development/delivery of new diagnostic and predictive medicine-based approaches).