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MANCHESTER CANCER RESEARCH CENTRE

ESMO Translational Research Fellowship

(10/2018 – 09/2019)

Dr Christoph Oing

FINAL REPORT

Host Institute: **The Manchester Cancer Research Centre, Manchester, United Kingdom**

Mentor: **Prof Dr Robert G Bristow, MD PhD**

Project title: **Hypoxia-driven Prostate Cancer Genomics (HYPROGEN)**

Home Institute: **University Medical Centre Hamburg-Eppendorf, Hamburg, Germany**

Introduction

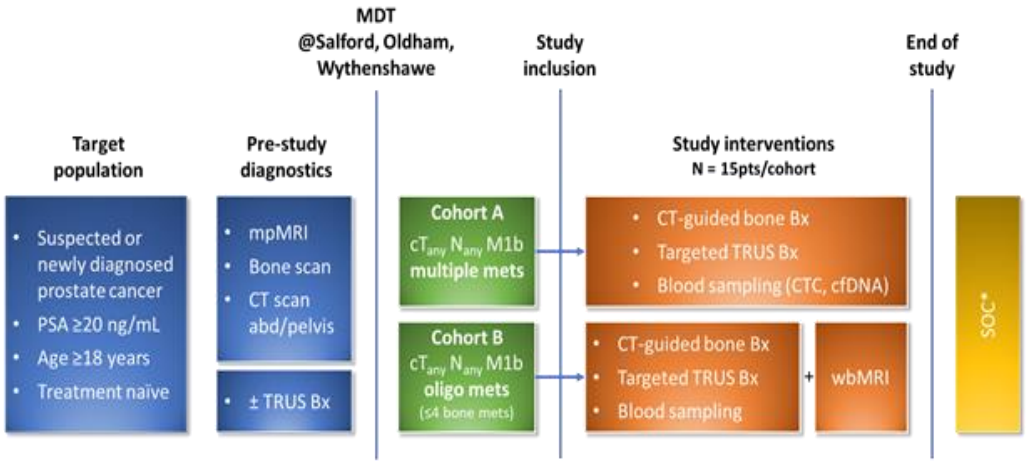
Around 47,700 patients will be diagnosed with PCa in the UK each year. About 15% present with primary M1 disease, which remains incurable despite remarkable improvements of treatment over the last decade. Therefore, novel targeted, biomarker driven treatment approaches are urgently needed. The tumour microenvironment is characterized by dynamic gradients of oxygen diffusion and consumption leading to sub-regions of hypoxia in about half of all solid tumours. Tumour adaptation to imbalanced oxygen supply and demand is associated with poor prognosis and elevated genomic instability, resistance to chemotherapy and radiotherapy, immune dampening, development of tumour stem cell protective niches and increased proclivity for distant metastasis, such as bone metastases [1, 2]. Consequently, cancer patients with hypoxic tumours, including PCa, have a dismal prognosis irrespective of applied treatment [3].

Hypoxia can select for cancer cells that are apoptosis-deficient, contain *TP53* mutations, and have increased genomic instability leading to a mutator phenotype [3]. Importantly, the Bristow lab previously reported that the co-presence of tumour hypoxia (based on mRNA signatures or needle electrode measurements) and genomic instability synergistically portend rapid relapse after primary treatment for PCa, supporting the concept that a hostile tumour microenvironment may select for or drive adaptation of a distinctive genomic profile and rapid failure due to occult metastases [4]. In line with this, a study from the West group of Manchester University showed that a prostate-specific hypoxia-associated 28-gene RNA expression signature portends poor prognosis [5], as was also shown for a different signature published by Ragnum et al. [6].

Ongoing Phase III studies are testing whether oligometastatic disease can be eradicated with local ablation in addition to best systemic care; but a mechanistic basis for further personalised intensified treatment in the systemic arms, e.g. by adding anti-hypoxia compounds for the most hypoxic tumors, is lacking. As hypoxia is scarce in normal tissues, targeting this characteristic of aggressive PCa will primarily kill cancer cells and augment the therapeutic ratio.

Rationale and Aim
The HYPROGEN translational research fellowship project assesses the complex tumour heterogeneity in *de novo* prostate cancer (PCa) patients with treatment-naïve, early M1b disease, both oligometastatic and widespread metastatic disease. The project aims are as follows: (1) Assess the differential genomic aberrations and gene expressional alterations in hormone-naïve primary PCa and paired skeletal metastases. (2) Determine whether co-existent intra-prostatic hypoxia, EMT and genomic instability is a co-factor in driving early spread. (3) Identification of co-existence of hypoxia in primary and metastatic tumour sites. (4) Assess the prevalence and origin of CTCs in early, aggressive (oligo-)metastatic disease. The HYPROGEN study still will constitute world's-first evidence for the co-existence of hypoxia, genetic instability and increased CTC abundance within the same patient and address the hypothesis that hypoxia drives genetic instability and tumour aggressiveness in lethal PCa. Ongoing Phase III studies are testing whether oligometastatic disease can be eradicated with local ablation in addition to best systemic care; but a mechanistic basis for further personalised intensified treatment in the systemic arms, e.g. by adding anti-hypoxia compounds for the most hypoxic tumors, is lacking. As hypoxia is scarce in normal, vital tissues, targeting this characteristic of aggressive PCa will most likely primarily kill cancer cells and augment the therapeutic ratio for patients with both, oligometastatic and widespread metastatic disease.

Experimental design
HYPROGEN is a a prospective, non-randomised, single-center, exploratory biomarker study for targeted tumour tissue sampling in men with newly diagnosed, untreated metastatic prostate cancer involving patients with either high (cohort A; widespread metastatic disease) or low, oligometastatic (cohort B; ≤ 4 bone metastases) burden of disease. This study establishes a comprehensive multidisciplinary collaboration between the Urology, Radiology, Pathology and Medical/Clinical Oncology Departments at The Christie NHS Foundation Trust and involves Christie-affiliated hospitals of the Greater Manchester region for patient identification, referral and study inclusion. The following flow chart shows the set-up of the HYPROGEN study:



*SOC, standard of care: Androgen deprivation ± Docetaxel or Abiraterone/prednisolone ± Radiotherapy of prostate, check study availability, i.e. STAMPEDE

Tissue samples from both the prostatic primary and a bone metastasis and a blood sample will be assessed. To yield highest possible biopsy accuracy and tissue amount and maximum procedure safety, prostate biopsy will be a TRUS-guided targeted transperineal biopsy and bone biopsy will be CT-guided using an automated drill. Moreover, to identify the imaging modality with the highest accuracy in detecting prostate cancer at an oligometastatic stage, an additional whole-body MRI will be applied to patients of study cohort B. Study participation will not affect the routine care for this stage of disease, which will start immediately after the biopsy visit. To identify hypoxic tumour tissue for further analysis, patients will be asked to ingest an oral formulation of pimonidazole hydrochloride (HCl) (Oral Hypoxyprobe^{TM}-1). Pimonidazole HCl is a marker for hypoxia in tumour tissue when ingested as an encapsulated solid. Following oral administration, pimonidazole distributes throughout the body where it covalently binds to normal and tumour tissues that have regions of low oxygen concentrations (pO_2 of ≤ 10 mmHg at 37°C). The tissue binding can be visualised by immunohistochemistry / light microscopy and respective tissue compartments will be separated by microdissection.

The following methodologies are being applied to the samples to answer the research aims as mentioned above:

(1) Assess the differential genomic aberrations and gene expressional alterations in hormone-naïve primary PCa and paired skeletal metastases.

Whole exome sequencing (WES) analysis will be performed and the results of the paired samples of the study patients will be compared in silico to ~500 patients with pT1-3 N0M0 disease from the CPC-GENE bioinformatics portal as a control set [7]. Moreover, to assess the gene expression profile of primary tumour and metastases, the whole RNA will be sequenced (RNAseq).

(2) Determine whether co-existent intra-prostatic hypoxia, EMT and genomic instability is a co-factor in driving early spread

Pimonidazole (PIMO)-labelled hypoxic areas of primary (if available) and metastatic tumour tissue will be micro-dissected and analysed by WES, RNAseq, and a targeted mRNA analysis to detect the Manchester 28-gene expression signature predicting for tumour hypoxia. Data pertaining to the co-occurrence in PIMO-positivity *in situ*, Copy Number Alterations, Single Nucleotide Variants and whole RNA expression profiles as well as the Manchester hypoxia 28-gene RNA signature will be compared.

(3) Identification of co-existence of hypoxia in primary and metastatic tumour sites

Results of PIMO-staining and the RNA expression hypoxia screening of the samples will be compared for each patient to identify co-existent hypoxia in primary PCa and paired bone metastases.

(4) Assess the prevalence and origin of CTCs in early, aggressive (oligo-) metastatic disease

In collaboration with the Manchester Centre for Cancer Biomarker Sciences (MCCBS; Professor Caroline Dive, Jonathan Tugham) blood samples will be used to assess the prevalence and origin of CTCs and ctDNA. To increase sensitivity of detection marker-independent platforms for CTC capture and analysis will be used. CTCs will be isolated and molecularly profiled to determine whether they originate from primary tumour and/or from metastases. Moreover, CTC genomes will be mapped to pimonidazole high and low primary/metastatic tumour regions to determine hypoxia (and hypoxia associated EMT) drives tumour cell dissemination.

Results, Conclusions and Future Perspectives

Due to staff changes during the study development phase and the complexity of study set up with regards to necessary adaption of patient referral pathways, harmonisation of the planned, multidisciplinary biopsy procedures, difficulties to safeguard the drug (tissue marker, pimonidazole) supply as well as the necessity to provide precautions for the impeding Brexit, the HYPROGEN study only very recently received final ethical approval from the Greater Manchester Research Ethics Committee and just now passed the various regulatory quality checks of The Christie's Research & Development Department. The study will finally start patient recruitment in February 2020. Fortunately, the study will be organised in collaboration with the Christie's Genitourinary Research Team.

A continued leading role for me within this research project has been assured by the project supervisor. To achieve this, I will go to Manchester regularly to ensure timely and proper conduct of the study, as well as for data analysis and project finalisation in the course of 2020/2021.

Moreover, based on the experiences from the cumbersome but finally successful setting up of the HYPROGEN study, I was successful in setting up a methodologically closely related research programme at my home institution in Hamburg, Germany, to explore the relationship between tumour hypoxia, genomic instability and nodal metastatic spread in systemically untreated synchronous or metachronous N+ prostate cancer patients called "DNA repair, hypoxia and genomic instability in metastatic prostate cancer" (DRAGOON).

Data obtained from both projects, HYPROGEN and DRAGOON will be merged and jointly analysed in a close collaboration with my ESMO translational research fellowship host Prof. Bristow and his Translational Oncogenomics Group at the Manchester Cancer Research Centre. DRAGOON has been approved and will be funded by the Mildred Scheel Foundation of the German Cancer Society.

In addition to the HYPROGEN study, several further research projects have been launched in Manchester with the support of Prof. Silke Gillessen, who got me involved in the Manchester Penile Cancer Research Group and the EORTC for a highly innovative clinical-translational study on muscle-invasive bladder cancer. Both projects are continuing, and I will stay involved by keeping my honorary research fellow post at the University of Manchester and The Christie NHS Foundation Trust.

List of Publications and Presentations Resulting from the Translational Research Project "HYPROGEN"
• Nil directly related to the Translational Research Project so far. 1-3 publications expected for 2020/2021 period.

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)
(1) TP53 and Prognosis in mCRPC Survival: Biology or Coincidence? Rebello RJ, **Oing C**, Gillessen S, Bristow RG. Clin Cancer Res. 2019 Mar 15;25(6):1699-1701. (2) Proteomic Comparison of Malignant Human Germ Cell Tumor Cell Lines. Bremmer F, Bohnenberger H, Küffer S, Oellerich T, Serve H, Urlaub H, Strauss A, Maatoug Y, Behnes CL, **Oing C**, Radzun HJ, Ströbel P, Balabanov S, Honecker F. Dis Markers 2019; 2019:8298524 (3) Awareness of predatory journals and open access among medical oncologists: results of an online survey. Richtig G, Richtig E, Böhm A, **Oing C**, Bozorgmehr F, Kruger S, Kiesewetter B, Zielinski C, Berghoff AS. ESMO Open 2019; 4:e000580. (4) Treatment of refractory germ-cell tumours with single-agent cabazitaxel: a German Testicular Cancer Study Group case series. **Oing C**, Hentrich M, Lorch A, Gläser D, Rumpold H, Ochsenreither S, Richter S, Dieing A, Zschäbitz S, Pereira RR, Bokemeyer C, Seidel C. J Cancer Res Clin Oncol 2019; *Epub ahead of print*.

Selection of Courses and Workshops Attended During the Fellowship
• The EORTC ECCO ESMO AACR Workshop on Methods in Clinical Cancer Research (MCCR), Zeist, NED, June 2019   ○ Awarded the "Most innovative protocol award" • ESMO Annual Meeting 2019, Barcelona, ESP

Acknowledgements
Prof. Robert Bristow, Director of the Manchester Cancer Research Centre, has become a true mentor for me. He is a great supporter to foster my academic career based on translational prostate cancer research in a larger scale. A close collaboration will continue and allow successful conduct of two highly original and comprehensive collaborative translational research projects, HYPROGEN and DRAGOON. Prof. Silke Gillessen has been also a great supporter during my stay in Manchester by getting me involved in clinical care within the GU Research Team at The Christie NHS Foundation Trust, where I was allowed to function as subinvestigator in numerous Phase II and Phase III studies for prostate and bladder cancer patients. With the support of my two mentors, the ESMO translational research fellowship has already become a great success, personally and professionally, with several ongoing research projects, which already foster my academic career as a clinician scientist.

Personal Statement
"The fellowship programme is a tremendous offer from ESMO to foster career paths of young medical oncologists. I am extremely grateful for ESMO to fund my translational research fellowship at the Manchester Cancer Research Centre, which gave me the opportunity to implement my own comprehensive research project together with Professor Robert Bristow and Professor Silke Gillessen as renowned and highly supportive mentors. The experience I gained and the network I was able to build will sustainably impact my future career in medical oncology and translational research. So thank you EMSO." *This statement has already been included in the anniversary brochure of the ESMO Fellowship Programme published during the ESMO Annual Meeting 2019.*

References
[1] Rankin EB, Nam JM, Giaccia AJ. Hypoxia: Signaling the Metastatic Cascade. Trends Cancer 2016; 2: 295-304. [2] Johnson RW, Sowder ME, Giaccia AJ. Hypoxia and Bone Metastatic Disease. Curr Osteoporos Rep 2017; 15: 231-238. [3] Taiakina D, Dal Pra A, Bristow RG. Intratumoral hypoxia as the genesis of genetic instability and clinical prognosis in prostate cancer. Adv Exp Med Biol 2014; 772: 189-204. [4] Lalonde E, Ishkanian AS, Sykes J et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. Lancet Oncol 2014; 15: 1521-1532. [5] Yang L, Roberts D, Takhar M et al. Development and Validation of a 28-gene Hypoxia-related Prognostic Signature for Localized Prostate Cancer. EBioMedicine 2018; Epub ahead of print. [6] Ragnum HB, Vlatkovic L, Lie AK, et al. The tumour hypoxia marker pimonidazole reflects a transcriptional programme associated with aggressive prostate cancer. Br J Cancer 2015; 112:382-90. [7] Fraser M, Sabelnykova VY, Yamaguchi TN et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature 2017; 541: 359-364.

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