

## **Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer.**

**Pasquale Rescigno**<sup>1,2,3</sup>, Daniel Nava Rodrigues<sup>1,2</sup>, David Liu<sup>4,5</sup>, Wei Yuan<sup>1</sup>, Nuria Porta<sup>6</sup>, Ines Figueiredo<sup>1</sup>, Diletta Bianchini<sup>2</sup>, Suzanne Carreira<sup>1</sup>, Ruth Riisnaes<sup>1</sup>, Susana Miranda<sup>1</sup>, Mateus Crespo<sup>1</sup>, Bora Gurel<sup>1</sup>, Maryou B. Lambros<sup>1</sup>, Penelope Flohr<sup>1</sup>, Claudia Bertan<sup>1</sup>, Matthew Clarke<sup>1</sup>, George Seed<sup>1</sup>, Adam Sharp<sup>1,2</sup>, Charles G. Drake<sup>7</sup>, Andrea Alimonti<sup>8</sup>, Emma Hall<sup>6</sup>, Eliezer M. Van Allen<sup>4,5</sup>, Johann S. de Bono<sup>1,2</sup>.

1. The Institute of Cancer Research, London, United Kingdom.
2. The Royal Marsden, London, United Kingdom.
3. Department of Clinical Medicine and Surgery, Department of Translational Medical Sciences, Azienda Ospedaliera Universitaria (AOU) Federico II, Naples, Italy.
4. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA.
5. The Broad Institute, Cambridge, Massachusetts, USA.
6. Clinical Trials and Statistics Unit, The Institute of Cancer Research, Sutton, United Kingdom
7. Columbia University Medical Center, New York, New York, USA.
8. Institute of Oncology Research (IOR), Bellinzona and Faculty of Biomedical Sciences, Università della Svizzera Italiana, Bellinzona, Switzerland.

**BACKGROUND:** Programmed cell death 1 (PD-1) blocking agents have become crucial components of the treatment of deadly diseases and have received FDA approval for the treatment of microsatellite instability-high (MSI-H) or mismatch repair-defective tumours (dMMR). However, their role in the treatment of prostate cancer remains largely unproven.

**METHODS:** Defective mismatch repair (dMMR) status was determined by either loss of mismatch repair protein expression on IHC or microsatellite instability (MSI) by PCR in 127 APC biopsies from 124 patients (Royal Marsden [RMH] cohort); MSI by targeted panel next-generation sequencing (MSINGS) was then evaluated in the same cohort and in 254 APC samples from the Stand Up To Cancer /Prostate Cancer Foundation (SU2C/PCF). Whole exome sequencing (WES) data from this latter cohort were analyzed for pathogenic MMR gene variants, mutational load, and mutational signatures. Transcriptomic data, available for 168 samples, was also performed.

**RESULTS:** Overall, 8.1% of patients in the RMH cohort had some evidence of dMMR, which associated with decreased overall survival. Higher MSINGS scores associated with dMMR, and these APCs were enriched for higher T cell infiltration and PD-L1 protein expression. In this same analysis, MSINGS of mCRPC biopsies from the PCF/SU2C cohort utilizing WES confirmed good correlation between WES-MSINGS and targeted panel MSINGS scores ( $r=0.73$ ,  $p<0.0001$ ). These data also identified four dominant mutational signatures matching previously described COSMIC mutational signatures: two MMRd-associated signatures, homologous recombination deficiency (HRD)-associated signatures and aging-associated signatures. High MSINGS scores associated with dMMR mutational signatures and MMR genes mutations. APC with dMMR mutational signatures overexpressed also a variety of immune cell, immune checkpoint, and T cell-associated transcripts including CD200R1, BTLA, PD-L1, PD-L2, ADORA2A, PIK3CG, and TIGIT.

Based on these data we have initiated a multi-stage open-label, single arm, phase II trial (PERSEUS1) utilizing a Simon Minimax design ( $p_0=0.20$ ;  $p_1=0.40$ ;  $\alpha=0.05$ ;  $\beta=0.1$ ). The primary objective is to determine the anti-tumour activity of the ICI pembrolizumab (200 mg IV 3-weekly) in mCRPC patients progressing on standard treatments deemed to have a high likelihood of ICI sensitivity based on a composite assay utilizing next generation sequencing and multiplex immunocytochemistry. Part A will recruit 45 patients, 24 in stage 1 and 21 in stage 2. The primary endpoint is tumour response.

Ineffectiveness will be concluded if  $\leq 5$  and  $\leq 13$  responses are seen in stage 1 or 2 respectively. If  $> 13$  responses are reported a subsequent (biomarker enriched) cohort will be pursued.

CONCLUSION: These data could impact immune target selection, combination therapeutic strategy selection, and selection of predictive biomarkers for immunotherapy in APC.

Conflict of interest: PR, DNR, WY, NP, IF, DB, SC, RR, SM, M. Crespo, BG, MBL, PF, CB, M. Clarke, GS, AS and JSdB are employees of the Institute of Cancer Research, which has a commercial interest in abiraterone. AA and EH have no conflict of interest to disclose. JSdB has served as a consultant/advisory member for Astellas Pharma, AstraZeneca, Bayer, Genmab, Genentech, GlaxoSmithKline, Janssen, Medivation, Orion Pharma, Pfizer, and Sanofi. EMVA has served as a consultant/advisory member for Genome Medical, Invitae, Tango Therapeutics, Illumina; has equity in Genome Medical, Tango Therapeutics, and Syapse; and has received research support from Novartis and Bristol-Myers Squibb. CGD has ownership interests in Compugen, Harpoon, Kleo, Potenza, and Tizona. He has served in a consulting role for Agenus, Dendreon, Janssen, Lilly, Merck, MedImmune, Pierre Fabre, and Roche/Genentech. He received research funding from Aduro Biotech, Bristol-Myers Squibb, and Janssen, and was an inventor on the following: US patent US8551481B2 for an anticancer vaccine composition comprising an anti-CD223 antibody and kit comprising an anticancer vaccine and an anti-CD223 antibody licensed from Johns Hopkins University St Jude's to Bristol-Myers Squibb.

We acknowledge funding support from Movember/PCUK CEO13-2-002, Stand Up To Cancer/Prostate Cancer Foundation to the International Prostate Cancer Dream Team, SU2C-AACR-DT0712, Prostate Cancer Foundation grants 20131017 and 20131017-1, Prostate Cancer Foundation Young Investigator Award (EMVA) and Department of Defense grant W81XWH (JSdB), Cancer Research UK, Experimental Cancer Medicine Centre grant funding from Cancer Research UK and the Department of Health, and Biomedical Research Centre funding to the Royal Marsden.