INVESTIGATION OF POTENTIAL THERAPEUTIC SOMATIC AND GERMLINE CALLS FROM TUMOUR/ESMO Open A222

Dissertation of glioblastoma is relatively rare but it could be more aggressive. Therefore, in vitro and in silico studies are accelerated in terms of the investigation of molecular characteristics of metastatic glioblastoma. The aim of this study, to investigate the expressed biomarkers of immigrant glioblastoma cells and potential therapeutic targets by using in silico methods.

Material and methods GEO (Gene Expression Omnibus) of the National Cancer Institute (NCI) provides as international public repository of research society, which archives and freely distributes microarrays, next generation sequencing and other forms of high-resolution functional genomic data. Data set enumerated as GSE76018, is used for this research from GEO data portal. The data was analysed by using R software for statistical calculations and graphs. The level of the gene expression, and the resulting data were standardised by calculating a z-score, the fold changes (log2FC). As a threshold, |log2FC| ≥ 2 and adjusted p-value was accepted as ≤0.05. Heat maps were drawn using a hierarchical clustering method in R-Bioconductor’s Heatplus library. Functional annotation tools and to understand biological meaning behind large list of genes analysed by using DAVID bioinformatics resources.

Results and discussions JAK-STAT pathway is found by using DAVID bioinformatics database. IFNA8, IL9, SOCS1, JAK3, CSH1, IFNL2 and IL12RB2 genes have played an active role in charge of protein coding Janus kinase (JAK) 2/signal transducer and activator of transcription (STAT) 3 pathway. So these genes affect the migration of glioblastomas and biomarkers is except to be effective at drug target. Sifalimumab and Rontalizumab connected to IFNA8, IL9 related to Enokizumab, also JAK3 linked to Tofacitinib.

Conclusion As a result of our investigation, JAK-STAT pathway, which is known to be an important mediator of tumour cell survival, growth and invasion in glioblastomas was, identified the migratory phenotype indicator. In this pathway, seven genes as therapeutic targets and related drugs potentially identified. Further in vitro and in vivo validation studies should be done for the confirmation for the provided biomarkers and therapeutic targets.

SOMATIC AND GERMLINE CALLS FROM TUMOUR/ NORMAL WHOLE GENOME DATA: BIOINFORMATICS WORKFLOW FOR REPRODUCIBLE RESEARCH

Introduction Whole-genome sequencing of cancer tumours is more a research tool nowadays, but going to be used in clinical settings in the near future to facilitate precision medicine. While large institutions have built up in-house bioinformatics solutions for their own data analysis, robust and portable workflows combining multiple software have been lacking, making it difficult for individual research groups to utilise the potential of this research field. Here we present Sarek, a robust, easy-to-instal workflow for identification of both somatic and germline mutations from paired tumour/normal/relapse samples.

Material and methods Sarek is open source and implemented in Nextflow; a domain specific programming language to enable portability and reproducibility. With the help of docker containers the versions of the underlying software can be maintained. Furthermore, with Singularity it is possible to run the workflow on protected clusters with no internet connexion.

The workflow starts from raw FASTQ files, and follows the GATK best practices to prepare the recalibrated files with joint realignment around indels for both the tumour and the normal data. Reads are alignment to the GRCh38 human reference in an ALT-aware settings using BWA, however, it is possible to assign other references. HaplotypeCaller and Strelka2 germline calls are collected for both the tumour and the normal sample, and Manta provides germline structural variants. The somatic variations are calculated by running MuTect2, Strelka and FreeBayes (and MuTect1 optionally). Somatic structural variants are delivered by Manta, and ASCAT estimates ploidy, tumour heterogeneity and CNVs.

The resulting variant call files are annotated by SnpEff and Ensembl-VEP. The annotated calls are further filtered and prioritised by our custom methods. During running the workflow quality control metrics are also calculated and aggregated by MultiQC.

Results and discussions Sarek was validated on a real dataset with known golden set of somatic mutations. In a real settings, whole-genome sequencing (WGS, 45–60x coverage) of patient-matched tumour and blood derived-DNA is being performed on a set of 80 paediatric brain tumour samples of the Swedish Childhood Tumour Biobank. The workflow helps to produce, filter, prioritise and characterise both germline and somatic variations.
Conclusion  Sarek is a portable bioinformatics pipeline for WGS normal/tumour matched samples, aiding precision medicine by improved subtyping and to gain novel functional insights in a reproducible framework.

Clinical Phase I – II Trials with Targeted Drugs and Novel Agents

**PO-513 USING MAPK AND PI3K SIGNALLING TO PREDICT PATIENT OUTCOME IN RESECTABLE STAGE IIIIB/C MELANOMA PATIENTS NEOADJUVANTLY TREATED WITH DAFRAFENIB AND TRAME tinib**

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**Introduction** In the recent decade, significant progress has occurred in the treatment of stage IV metastatic melanoma, which is now being applied to patients with earlier stages of disease. Targeted therapies that combine Dabrafenib (BRAF inhibitor) and Trametinib (MEK inhibitor) have achieved a median progression-free-survival of 9.4 months in stage IV patients. However 50% of the patients on the combination therapy progress at 9–10 months. This can occur through MAPK reactivation of MAPK/ERK signalling and the PI3K/ AKT/mTOR pathway.

**Material and methods** In our study, we performed multispectral immunofluorescent staining on 26 Stage III metastatic melanoma patients who received neoadjuvant treatment with combination Dabrafenib and Trametinib. Patients were categorised into complete responder (CR) or non-complete responder (non-CR) based on their pathological response. Longitudinal biopsies were taken before therapy (PRE) and early during treatment (EDT) (day 4–7), with a full lymph node dissection at 12 weeks. PRE, EDT and 12 week (non-CR only) were assessed using quantitative pathology for phosphorylated-ERK (p-ERK), p-AKT and Ki-67 positivity in melanoma cells.

**Results and discussions** In CRs, pERK decreased by 97% from PRE to EDT, whilst non-CRs decreased by 89%. Expression of pERK increased by 61% from EDT to the 12 week dissection in non-CR patients. There was no change between PRE and EDT in pAKT expression in either CR and non-CR patients; however in non-CRs, pAKT expression increased by 37% from EDT to 12 weeks. In CRs, Ki67 expression decreased by 85% from PRE to EDT (p=0.019), with higher ki-67 expression in non-CR than CR patients at baseline (23% and 11% respectively, p=0.069).

**Conclusion** This study highlights that patients who achieve a CR on Dabrafenib+Trametinib therapies have a significant decrease in their Ki67 expression from their PRE to EDT biopsies.

**Introduction** Innovations in both targeted and immunotherapy (IT) of metastatic melanoma have led to improved responses in a considerable number of patients. For both types of therapies, resistance remains a formidable challenge. For IT, knowledge on the mechanisms behind resistance is an urgent and unmet need for better patient stratification. Gene expression signatures to predict response in baseline samples could help to better stratify patients. It is expected that both tumour cell intrinsic signals and stromal signals play a role. However, gene expression signatures are typically derived from RNA-sequencing data from patients’ tumours, where the distinction between stromal and tumour signals cannot be made. Here, we take advantage of our melanoma PDX platform [Kemper et al. Cell Reports 2016] to dissect the stromal and tumour cell intrinsic signals and relate these to the response to immune checkpoint blockade.

**Material and methods** RNA sequencing was performed on 95 PDX tumours derived from metastatic melanoma [Kemper et al. Cell Reports 2016]. Gene expression data and clinical information of patients treated with anti-PD1 and anti-CTLA4 were downloaded [Hugo et al. 2016 & Van Allen, 2015]. Additionally, we performed RNA sequencing on samples of patients treated with the combination of anti-CTLA4 and anti-PD1 [Blank et al. submitted].

**Results and discussions** We found that tumour cell-intrinsic and stromal gene expression signatures differentially predicted response to anti-PD1 and anti-CTLA4 immune checkpoint inhibition. Furthermore, a combination of signatures predicts response in patients treated with the combination of anti-PD1 and anti-CTLA4.

**Conclusion** Our PDX platform provided the possibility to computationally dissect the gene expression signals from tumour cells and stromal tissue. These stromal and tumour intrinsic gene expression signatures differentially predict response to anti-PD1 and anti-CTLA4 in baseline samples which can be used for better patient stratification.