negativity in breast tumours and demonstrated their frequent expression in tumours with higher nuclear grade. The expression of cancer/testis antigens in ductal carcinoma in situ is not studied so extensively as in invasive breast cancer.

**Material and methods** This retrospective study included archived paraffin-embedded specimens from 83 patients diagnosed with DCIS in the period between 2007 and 2014, a mean follow up time for local recurrence was 6.5 years. Antigens multi-MAGE-A, MAGE-A1, MAGE-A10 and NY-ESO-1 and were demonstrated by immunostaining. TILs were determined on all sections together with the histopathological variables of DCIS.

**Results and discussions** All tested antigens showed association (positive or negative) with histopathological parameters. Expression of MAGE-A1 was significantly associated with cytoplasmic staining (p=0.007). Simultaneously cytoplasmic and nuclear staining was in statistically significant positive correlation with local recurrence (p=0.005) and central necrosis (p=0.016) and in negative correlation with expression of ER receptors (p=0.003) and PR receptors (p=0.009). Antigen MAGE-A10 was significantly associated with tumor-infiltrating lymphocytes (p=0.05). The additional analysis of TILs showed statistically significant positive correlation with grade (p=0.023), and central necrosis (p<0.001), and negative with tumour size (p=0.623), ER receptors (p=0.003) and PR receptors (p=0.027).

**Conclusion** Cancer/testis antigens from MAGE family (MAGE-A1, multi-MAGE-A and MAGE-A10) and NY-ESO-1 correlate with histopathological predictive variables of DCIS. The expression of antigen MAGE-A10 could have an important role in treatment of patients with negative histopathological predictive variables, but further analysis is required. Simultaneous cytoplasmic and nuclear protein expression of MAGE-A family and NY-ESO-1 cancer-testis antigens represent an independent marker for local recurrence. Cancer/testis antigens are not perfect indicators of invasiveness for DCIS, but in combination with others histopathological predictive variables they can be used as a guidance for better treatment of this patients. But, this is the small study and further larger studies are necessary to confirm our findings.

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**Additional abstracts**

**PO-342 INTEGRATING COPY NUMBER ANALYSIS AND TUMOUR DNA METHYLATION PROFILING**

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10.1136/esmoopen-2018-EACR25.854

**Introduction** The cancer epigenome is a subject of intense research to identify new therapeutic targets and markers for diagnosis. Independent reports have showed that the cancer methylome is heterogeneous and that high levels of intratumour heterogeneity (ITH) correlate with poor prognosis. In this project, we aim to perform copy number profiling from bisulfite sequencing data and integrate allele specific copy number and purity estimates to deconvolve the pure tumour methylome and better our understanding of ITH and tumour evolution.

**Material and methods** As part of the TRACERx study (TRACKing Cancer Evolution through therapy (Rx)), we analyse 38 cases of non-small cell lung cancer (NSCLC) with multi-region whole exome (WES) and reduced-representation bisulfite (RRBS) sequencing of the primary tumour for a total of 169 samples.

We developed a novel bisulfite sequencing module for ASCAT (allele-specific copy number analysis of tumours), a method originally designed for SNP array data which computes the copy number profile of tumour cells, accounting for normal cell admixture and tumour aneuploidy.

**Results and discussions** Tumour purity and copy number estimates were obtained using RRBS and validated using WES. We show that local copy number affects methylation rates extracted from the RRBS data in a way that can be modelled. If we have a CpG that is methylated in the tumour and unmethylated in the normal contaminating cells, the observed fraction of methylated reads in the bulk increases as a function of copy number given sample purity. Vice versa, the observed methylation rate will decrease with increasing tumour copy number when the tumour is unmethylated but the normal is methylated.

Leveraging the observed relationship between methylation rate, copy number and tumour purity, we can extract the pure tumour methylation profiles from the RRBS data. The pure profiles allow for identification of differentially methylated regions (DMRs) between cancer and normal cells as well as between cancer subclones. We then reconstruct the evolutionary history of the tumour by hierarchical clustering of the DMRs. The obtained phylogenetic trees are validated by performing SNV clustering on the WES data.

**Conclusion** We show that epigenetic ITH is present in NSCLC and integrate the cancer epigenome with other layers of ‘omics data to deepen our understanding of tumour evolution. Next, we aim to provide timing estimates for DMRs and identify potential drivers based on event recurrence and impact on gene expression from RNA-Seq data.

**PO-343 THE MICRORNA-146B-IRAK1 SIGNALLING AXIS IN TUMOUR RECURRENCE OF PAPILLARY THYROID CANCER**

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10.1136/esmoopen-2018-EACR25.855

**Introduction** Papillary thyroid cancer (PTC) is the most common tumour subtype of thyroid cancer. However, not all PTCs are responsive to current surgical and radiiodine treatment. The well-established clinical prognostic factors include tumour size, lymph node/distal metastasis, and extrathyroidal invasion. The RET/PTC-RAS-BRAF linear molecular signalling cascade is known to mediate PTC pathogenesis. However, whether presence of BRAF mutation, the most common genetic alteration in PTC, can affect PTC behaviour and prognosis is controversial. MicroRNAs (miRNAs) have been labelled as promising
molecular prognostic markers in several tumour types. However, the underlying mechanism of miR-146b in relation to its oncogenic behaviour in PTC and its putative targets remain to be fully elucidated.

**Material and methods** We used genome-wide microarray, computational analysis and 3 UTR reporter gene assays to identify IRAK1 as a miR-146b target gene. In *vitro* gain/loss-of-function experiments were fur- ther performed to determine the effects of IRAK1 on proliferation, colony formation, and wound- healing in PTC cancer cell lines. Expression levels of miR-146b and IRAK1 of 50 cases of PTC and its adjacent normal thyroid specimens were assessed via quantitative real-time polymerase chain reaction.

**Results and discussions** Microarray expression profile revealed that the mRNA level of IRAK1 gene was down- regulated by miR-146b. The 3 UTR of IRAK1 mRNA was found to be a molecular target of miR-146b posttranscriptional repression in BCPAP cells by reporter gene assays. MiR-146b promoted the migration and proliferation of PTC cells by downregulating IRAK1 expression, whereas restoration of IRAK1 expression reversed this effect. In addition, the expression of IRAK1 mRNA was significantly lower in PTC clinical tissue samples than normal adjacent thyroid specimens and showed a strong inverse correlation with the expression of miR-146b in PTC specimens. Our results demonstrated that IRAK1 is a direct target of miR-146b and has functional roles to inhibit various aggressive PTC cell activities.

**Conclusion** In conjunction with current therapeutic regimens, targeting the miR-146b-IRAK1 axis not only may provide a potential approach for PTC management but also serve as a unique supplemental tool for diagnosis and predicting prognosis for PTC in the near future.

### PO-344 MIR-302B AS ADJUVANT THERAPEUTIC TOOL TO IMPROVE CHEMOTHERAPY EFFICACY IN HUMAN TRIPLE NEGATIVE BREAST CANCER

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**Introduction** MiRNAs are a class of non-coding regulatory RNAs playing key roles in different biological processes including cancer. Triple-negative breast cancer (TNBC) accounts for 15%–20% of all breast cancer cases, with the worst outcome of all subtypes. For TNBC, still lacking targeted therapies, the only therapeutic option is chemotherapy. MiRNAs can modulate chemotherapy response by affecting DNA repair, cell cycle progression, apoptosis and also tumour microenvironment. Macrophages constitute a major component of the immune microenvironment of cancer and pro-tumour M2 macrophages have been associated with response to chemotherapeutic treatments. Here, we investigated the potential of miR-302b as a therapeutic tool to enhance cisplatin sensitivity in a TNBC mouse model and which pathways are involved in this mechanism both in tumour cells and microenvironment.

**Material and methods** TNBC cells were injected into the mammary fat pad of female SCID mice and then treated with lipid nanoparticles containing miR-302b or cel-67 control, alone or in combination with cisplatin. Gene expression profile on collected tumours was performed by microarray. ITGA6 expression was assessed on tumour samples and siRNA transfection was performed to evaluate the cisplatin response. Tumour sections were stained with anti-arginase 1 (M2 marker) to assess the number of M2 macrophages, and luciferase assay was used to evaluate Irf4 (M2 marker) as a direct target of miR-302b.

**Results and discussions** Our results show that combination of miR-302b with cisplatin significantly impaired tumour growth in comparison with control cel-67. Gene expression profile identified ITGA6 as a regulatory target of miR-302b and cisplatin activity. Indeed, ITGA6 expression is down-modulated in mice treated with miR-302b plus cisplatin compared with control mice. Furthermore, TNBC cell lines increase their cisplatin sensitivity upon ITGA6 silencing. These data confirm the role of ITGA6 in cisplatin response mediated by miR-302b. Moreover, in xenograft tumours collected from the *in vivo* miR-302b delivery experiment, we observed a reduced number of M2 macrophages in the tumour microenvironment and gene expression confirm immune system modulation. Finally, luciferase assay validate Irf4, a key gene involved in M2 recruitment, as a direct target of miR-302b.

**Conclusion** Our data demonstrate that miR-302b can be exploited as a new therapeutic tool to improve the response to chemotherapy, modulating ITGA6 expression in tumour cells and M2 recruitment in tumour microenvironment.

### PO-345 IDENTIFICATION OF AR-MODULATORY MICRORNAs FOR PROSTATE CANCER PROGRESSION AND THERAPY

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**Introduction** Prostate cancer (PCa) is the most common type of cancer affecting the male population. It is an androgen dependent malignancy that initially responds well to androgen ablation therapy. However, despite hormonal treatment, castrate resistant prostate cancer eventually emerges. MicroRNAs (miRs), a class of small non-coding RNAs, modulate gene silencing through inhibition of translation and mRNA degradation. MiRs and/or miR inhibitors that reduce AR activity represent a promising therapeutic strategy, therefore a high-throughput miR inhibitor screen was performed in PCa cell lines, expressing a luciferase-based AR reporter. We sought to validate and characterise AR-modulatory miRs and identify their targets in PCa cell lines.

**Material and methods** Prostate cancer cell lines that stably expressed an AR reporter element were transfected with specific miR inhibitors and mimics. The effect of miR modulation on potential alteration of AR activity was investigated (through a luciferase assay), as well as the potential impact on cell growth by an SRB assay and on apoptosis, using a caspase 3/7 assay. Real-time qPCR and western blot assays were performed, to check the potential effect of miR manipulation on mRNA and protein levels of AR and miR specific target genes.

**Results and discussions** MiR-1271–5 p inhibitor significantly reduced AR activity and increased apoptosis in a castrate resistant cell line and the opposite effect was seen with the use of the mimic. MiR-1271–5 p inhibitor significantly