anti-inflammatory pathway mediated by IL-10, TGF-b, platelet-activating factor, and prostaglandin E2 (PGE2) suppresses inflammation (6). During radiation the high levels of DNA damage results in the release of a high concentration of pro-inflammatory "damage-associated molecular patterns" (DAMPS) including oxidized DNA, adenosine triphosphate (ATP) heat shock proteins (HSPs) and high -mobility group box 1 (HMGB1) (7), leading to upregulation of inflammatory pathways through activation of TLR's and triggering of pro-inflammatory cytokine cascades (8). This acute inflammatory reaction contributes to several of the hallmarks of acute radiation toxicity, including erythema, ulceration and oedema (9). Finally, chronic inflammatory responses induced by radiotherapy contribute to radiation fibrosis; a result of imbalance in the creation and destruction of extracellular matrix components mediated by the upregulation of pro-inflammatory cytokines (TNFα, IL1, IL-4, IL6) and fibrogenic cytokines (TNFβ) (10). **Summary** Our understanding of the molecular biology of radiation toxicity continues to evolve but is increasingly seen to be the result of the complex interplay of dysregulated DDR, immune and inflammatory responses. These pathways will provide a rich source of future therapies to increase both the efficacy and safety of radiotherapy treatment. References De Ruysscher D, Niedermann G Burnet N Siva S, Lee A, Hegi-Johnson F. Radiotherapy Toxicity, Nature Reviews Disease Primers (2019) 5:13 Lomax M, Folkes L, O'Neill P. 2013. Biological consequences of radiation induced DNA damage: relevance to radiotherapy. Clin Oncol (R Coll Radiol). 25:578—585. Morgan, M. A. & Lawrence, T. S. Molecular pathways: overcoming radiation resistance by targeting DNA damage response pathways. Clin Cancer Res 21: 2898-2904 (2015). Qiu, W. et al. PUMA regulates intestinal progenitor cell radiosensitivity and gastrointestinal syndrome. *Cell Stem Cell* 2, 576—583 (2008) Vanpouille-Box, C. et al. DNA exonuclease Trex1regulates radiotherapy-induced tumour immunogenicity. *Nat. Commun.* 8, 15618 (2017). Chung EY, Kim SJ, Ma XJ. 2006. Regulation of cytokine production during phagocytosis of apoptotic cells. Cell Res; 16: 154-161. Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, T€uting T, Hartmann G, Barchet W. 2013. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. Immunity 39:482—495. Piccinini A, Midwood K. 2010. DAMPening inflammation by modulating TLR signalling. Mediat Inflamm. 2010:672395. Sprung et al 2015. Immmunological markers that predict radiation toxicity. Cancer Lett 368:191-197 Yamada M, Kubo H, Ota C, Takahashi T, Tando Y, Suzuki T, Fujino N, Makiguchi T, Takagi K, Suzuki T. 2013. The increase of microRNA-21 during lung fibrosis and its contribution to epithelial-mesenchymal transition in pulmonary epithelial cells. Respir Res. 14:95. **Keywords:** radiotherapy, toxicity, Radiobiology

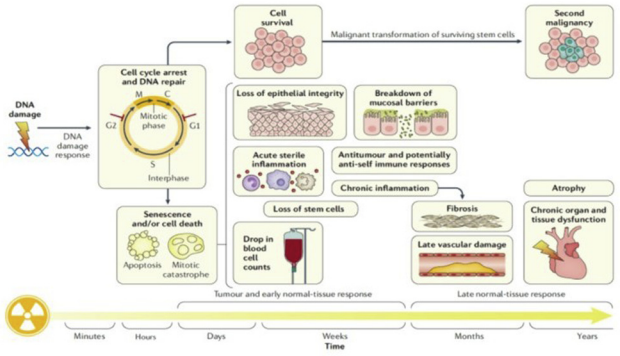

Figure 1: Cellular and Tissue Damage to Radiation Therapy. Taken from De Ruysscher D, Niedermann G ... Hegi-Johnson F. Radiotherapy Toxicity, Nature Reviews Disease Primers (2019) 5:13 (1)

ES17.01
Mesothelioma Evolution

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Malignant pleural mesothelioma (MPM) is mostly caused by prior exposure to asbestos fibres. It has a long but variable latency period following exposure, with a median of around 40 years but a range between 20-70 years, possibly reflecting the length and level of asbestos exposure as well as other environmental and genetic causes. Upon diagnosis, there are limited treatment options, and median survival time is a year, although, again, this is highly variable. Understanding the genetic events in the mesothelium between asbestos exposure and diagnosis of MPM is important for two reasons. Firstly, it will inform the biology of MPM tumour growth and potentially highlight different environmental and genetic factors that cause the variation in latency. Secondly, it will help identify key early driver mutations in MPM informing biology and candidate changes for developing approaches for early detection of the cancer. Evolutionary genetics is based on inferring past events from current genetic/genomic sequences. It has long been recognised that the development of cancer is an evolutionary process, and the availability of large amounts of DNA sequence data have facilitated an understanding of evolution of tumours using methods mostly borrowed from evolutionary genetics. One powerful approach compares a matched tumour and normal genome, infers the somatic mutations in the tumour, and uses the ratio of mutations that change an amino acid to mutations that don't change an amino acid (dN/dS ratio) across all genes to identify particular genes that have been positively selected during tumour evolution. This approach can also shed light on overall evolutionary processes that have occurred in the tumour. Genomic sequences from multiple regions of the same tumour not only emphasise the molecular heterogeneity of tumours but allow an explicit phylogenetic tree of the evolution of the tumour for each patient, distinguishing somatic mutations that happened early in the tumour's evolution (and are therefore present throughout the tumour) from those that happened late in the tumour evolution. Here, I report preliminary findings from a British Lung Foundation/Mesothelioma UK-funded project entitled MEDUSA — Mesothelioma Evolution: Deciphering drUgable Somatic Alterations as potential targets for synthetic lethal therapy. This project uses multiregional sampling of MPMs, together with matched whole blood, to infer a phylogenetic tree of MPMs. The preliminary data presented focuses on the first 20 patients, with between 4 and 5 regions of the tumour analysed per patient. Using whole exome sequencing, the project aims to identify truncal changes, that is, mutations that happened early in the tumour and are present throughout the tumour that can be potential targets for drugs, with the aim of developing personalised, effective tumour treatment for each patient. We focus on copy number changes (deletions and duplications of genes) and confirm that MPM is highly heterogeneous with extensive copy number changes in the genome. We focus on truncal copy number changes in ~20-25% of patients affecting the known mesothelioma tumour suppressor genes *BAP1*, *MTOR*, *CDKN2A* and *SETD1*. Distinguishing patients that have truncal copy number changes in these genes, in contrast to those patients with copy number changes in the terminal branches of the evolution of the tumour, helps to tailor individualised drug therapies. Our approach emphasises the importance of multiregional sampling of tumours to account for MPM heterogeneity. For example, by sampling the posterior costophenic angle of these MPM from these 20 patients, we would find 10 deletions of *CDKN2A*, of which only 5 are truncal, with the other five localised to only part of the tumour. Multiregional genomic analysis and evolutionary genetics approaches can illuminate the history of a tumour and have the potential to guide

therapy. They also provide the framework for follow-on studies in a patient, such as analysing the origin of metastases and identifying the effects on the tumour of treatment. The extra information provided by multiregional sampling supports the idea that this approach should become routine in tailoring the treatment to the tumour in MPM. **Keywords:** evolution, Mesothelioma, Genomics

ES17.02
Molecular Heterogeneity

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Malignant Pleural Mesothelioma (MPM), a rare thoracic tumor strongly linked to asbestos exposure, is one of the most aggressive cancer with a very poor prognosis. Clinical trials have highlighted MPM diversity in terms of prognosis and patients' response to anti-cancer agents, suggesting an underlying tumor heterogeneity. As current treatment options are rarely curative, a better characterization of inter and intra-tumor heterogeneity is essential for the identification of new therapeutic strategies, and for the implementation of precision medicine with the aim to improve the cure to this dreadful cancer. The first level of inter-tumor heterogeneity in MPM is histologic with three main histologic types i.e. epithelioid, sarcomatoid and biphasic. The latter is also an evidence of intra-tumor heterogeneity as the biphasic histologic type is a mix of variable proportion of epithelioid and sarcomatoid tumor cells. The histologic heterogeneity is even more complex with the characterization of several histologic subtypes (1). Large-scale omics and NGS (Next Generation Sequencing) studies also highlighted MPM heterogeneity at the molecular level. MPM show a complex pattern of chromosomal abnormalities and mutations, so it is difficult to take into account this molecular heterogeneity of MPM solely on the basis of chromosomal or genetic alterations. We and others, using unsupervised hierarchical clustering based on transcriptomic or integrated multi-omics data, defined molecular classification in 2 and 4 tumor subtypes (2-4). These molecular subtypes are related to histology and associated to prognosis, to specific mutations in genes such as *BAP1* and to the deregulation of specific signal pathways such as epithelial-mesenchymal transition (EMT). Smaller and highly homogeneous subtypes were also defined by taking into account molecular subtypes and mutation profiles such as the one characterized by a double inactivation in the two tumor suppressor genes related to Hippo signal pathway, *NF2* and *LATS2* (5). Interestingly, based on preclinical studies, a potential target therapy has been proposed for this subtype illustrating the interest to define homogenous tumor subtypes in order to develop new therapeutic approaches. However, these molecular classifications in subtypes have some limitations. First, they take into account only inter-tumor heterogeneity but not intra-tumor heterogeneity, which is poorly described at the molecular level in MPM (6). Second, a meta-analysis comparing all molecular subtypes obtained by unsupervised hierarchical clustering of several different transcriptomic dataset highlighted only two main subtypes, which are highly correlated in all datasets. Apart from these two opposite subtypes corresponding to pure epithelioid and sarcomatoid phenotypes, intermediate subtypes could simply reflect various cut-offs of a continuum combining epithelioid and sarcomatoid entities, which could be better defined using molecular gradients (7). For these reasons with the aim to better characterize MPM molecular heterogeneity, we used a deconvolution method that decomposes the MPM transcriptomic profile of each tumor as a combination of epithelioid and sarcomatoid components. We determined the proportion of these epithelioid and sarcomatoid components (E.score and S.score, respectively) in large series of tumors. These two opposite histo-molecular gradients were related to histology types and to subtypes of MPM molecular classification (7). The underlying oncogenic pathways driving the establishment of the epithelioid and sarcomatoid related cell entities were specified. Integration of transcriptome, methylome and miRNome data showed the strong contribution of epigenetic regulation. We also highlighted the link between the histo-molecular gradients and the tumor microenvironment and the immune contexts. A strong positive correlation was observed between the S.score and the infiltration of T lymphocytes, monocytes, fibroblasts and endothelial cells, while the E.score was linked to natural killer cells infiltration and complement pathway. These results suggested the presence of an adaptive immune response in tumors with a high S-score and of an innate immune response in tumors with a high E-score. The S.score was also strongly correlated with high expression of most immune checkpoint inhibitors, including *CD274* (*PDL1*) and *CTLA4* (7). More importantly, we highlighted the potent clinical impact of histo-molecular gradients on prognosis and on personalized therapeutic strategies in MPM. First, we showed that the S.score has a strong prognostic value, higher than histologic and molecular classifications. Second, our data supported that these histo-molecular gradients might be used to guide therapeutic strategies such as targeted therapies by performing preclinical studies. Third, the strong correlation of the S.score with T lymphocytes infiltration and immune checkpoint inhibitors expression supports that a high S.score could be predictive of immunotherapy based on anti-PDL1 and anti-CTLA4 inhibitors (7). Prediction of patients responding to these inhibitors is particularly important given the recent promising results of this immunotherapy for some MPM patients (8). More recently, we have performed a genetic profiling, focusing on the main key genes altered in mesothelial carcinogenesis, of a large collection of MPM with complete clinical annotations and well-characterized for heterogeneity using current available tumor classifications. The unpublished results provided a comprehensive overview of the genetic landscape of MPM taking into account the histologic and molecular heterogeneities. **References:** 1. Husain A. N. *et al.* Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2013, *137*: 647-667. 2. Bueno R. *et al.* Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet. 2016, *48*: 407-416. 3. de Reynies A. *et al.* Molecular classification of malignant pleural mesothelioma: identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. Clin Cancer Res. 2014, *20*: 1323-1334. 4. Hmeljak J. *et al.* Integrative Molecular Characterization of Malignant Pleural Mesothelioma. Cancer Discov. 2018, *8*: 1548-1565. 5. Tranchant R. *et al.* Co-occurring mutations of tumor suppressor genes, LATS2 and NF2, in malignant pleural mesothelioma. Clin Cancer Res. 2017, *23*: 3191-3202. 6. Oehl K. *et al.* Heterogeneity in Malignant Pleural Mesothelioma. Int J Mol Sci. 2018, *19*. 7. Blum Y. *et al.* Dissecting heterogeneity in malignant pleural mesothelioma through histo-molecular gradients for clinical applications. Nat Commun. 2019, *10*: 1333. 8. Scherpereel A. *et al.* Nivolumab or nivolumab plus ipilimumab in patients with relapsed malignant pleural mesothelioma (IFCT-1501 MAPS2): a multicentre, open-label, randomised, non-comparative, phase 2 trial. Lancet Oncol. 2019, *20*: 239-253. **Keywords:** Molecular classification, Histo-molecular gradient, tumor heterogeneity

ES17.03
Heterogeneity & Immune Checkpoint Expression

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Mesothelioma is a spatially complex malignancy. Morphologic heterogeneity is commonly identified, especially with surgical resection that can unmask distinct histologic components across sites of disease. Molecular analyses of *HUMARA* methylation patterns on the X-chromosome, *CDKN2A* deletions, and single nucleotide variants from multiple tumor sites all suggest that mesothelioma is polyclonal with multiple genetic subclones within each tumor. Temporal histologic heterogeneity has also been reported with sarcomatoid differentiation