Interim results of a Phase 1b/2 study of PV-10 and anti-PD-1 in advanced melanoma

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PV-10 (rose bengal disodium) is a small molecule oncolytic immunotherapy in development for solid tumors, where intralesional injection can yield immunogenic cell death and tumor-specific reactivity in circulating T cells. It has been administered as a single agent to 130 cutaneous melanoma patients in Phase 1 and 2 and 180 patients in expanded access, and is in Phase 3 for locally advanced cutaneous melanoma (Stage IIIB–IV M1a, NCT02288897).

Study PV-10-MM-1201 (NCT02557321) is a Phase 1b/2 study of PV-10 in combination with anti-PD-1 (pembrolizumab) for patients with advanced melanoma (Stage IIIC-IV M1c). Patients must have at least 1 injectable lesion and be candidates for pembrolizumab. In Phase 1b patients receive combination treatment q3w for 5 cycles then pembrolizumab alone for up to 24 months; the primary endpoint is safety and tolerability with objective response rate (ORR) and progression free survival as key secondary endpoints (assessed via RECIST 1.1 after 5 cycles then q12w).

Full accrual for Phase 1b was reached in April 2018, with an intent-to-treat (ITT) population of 20 Stage IV and 3 Stage IIIC/IIID patients (median age 70 years, range 28-90) receiving at least 1 dose of PV-10 and pembrolizumab. All Treatment-Emergent Adverse Events (TEAEs) were consistent with established patterns for both drugs, with no significant overlap of AEs or unexpected toxicities; Grade 1-2 AEs attributed to the combination were observed in 4 patients. Most patients had extensive uninjected tumor burden, and complete response (CR) was observed in non-injected visceral disease, including lung and liver metastases. Preliminary efficacy data on the first 10 patients were positive, with 50% ORR and 10% CR.

Acceptable safety and tolerability of the combination were observed with no unexpected safety issues. Interim efficacy and updated safety data for the full ITT population will be presented.

Hypoxia and Uveal Melanoma: effects of hypoxia on tumour formation and metastatic potential

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Uveal melanoma (UM) is a rare but aggressive ocular cancer affecting approximately 5 individuals per million per year. Despite effective treatment of primary tumours through radiation therapy and surgery, around 50% of UM patients develop metastases, primarily in the liver. These are generally fatal within 12 months following detection. There is currently no recognised adjuvant therapy for UM, and resection of liver metastases can be performed in relatively few patients. The biological pathways controlling UM metastasis are poorly understood, however it is well established in other tumours that hypoxia enhances vascularisation and acquisition of a phenotype resulting in cell mobility and metastasis. This study investigates the role of hypoxia in tumour formation and metastasis using the chick embryo chorioallantoic membrane (CAM) assay in UM.

Multiple UM cell lines were grafted on the chick embryo CAM and assessed for tumour nodule forming efficiency and metastatic potential. OMM1 and 92.1 UM cell lines formed tumour nodules on the CAM when exposed for 72hrs to either both normoxic and hypoxic conditions, which was also characterised by chick blood vessel recruitment into the tumour mass. Spontaneous metastasis to the liver was observed for both cell lines under normoxic conditions, though only 92.1 cells have been confirmed to form metastases under hypoxic conditions. Indeed, hypoxia pre-treatment in OMM1 cells caused a decrease nodule formation.

These data demonstrate the utility of the chick embryo model to study UM metastasis. Further experiments explore the *in vitro* effects of hypoxic conditions on the expression of hypoxia inducible factor 1α (HIF-1α), as well as changes in UM cell proliferation.

AGING INDUCES METABOLIC REWIRING OF FIBROBLASTS AND MELANOMA CELLS TO DRIVE THERAPY RESISTANCE

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"Aged" melanoma patients (>55 years old) have poorer prognosis and reduced response rates to targeted therapy relative to "young" patients (<40 years old) for reasons that are not completely understood. Here, we report that melanoma cells in an aged microenvironment accumulate and depend on lipids for therapy resistance. The accumulation of lipids occurs by two distinct mechanisms; 1) an elevated intake of aged fibroblast-derived fatty acids, as well as 2) an increase in intrinsic melanoma fatty acid biosynthesis. Our data indicate that aged fibroblasts significantly deplete bioavailable glucose in the aged microenvironment, driving melanoma cells to increase the FATP2 transporter for increased fatty acid intake. Additionally, secretome analysis of young and aged dermal fibroblasts identified >5-fold higher levels of insulin-like growth factor binding protein 2 (IGFBP2) in aged fibroblast secretomes. IGFBP2 functionally triggers an IGF1R-PI3K-dependent fatty acid biosynthetic program in melanoma cells, eliciting melanoma fatty acid dependence. FATP2 also serves a role in the conversion of free long-chain fatty acids into fatty acyl-CoA esters, and within our model, FATP2 is critical for IGFBP2-dependent fatty acid synthesis. FATP2 is upregulated in melanoma cells grown in the aged microenvironment *in vitro* and *in vivo*. Pharmacologically inhibiting the FATP2 transporter abrogated tumor growth and extended survival in aged immune-competent mice. Our data reveals the aged microenvironment changes the metabolic activity of melanoma cells, making them more aggressive and therapy resistant.

The landscape of driver mutations in cutaneous melanoma
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The sensitive and specific detection of positive selection on somatic mutations in sun-exposed melanomas is challenged by an exceptionally high rate of passenger mutations induced by ultraviolet radiation. To achieve a greater sensitivity, we pooled somatic variant calls from five whole genome and whole exome sequencing studies, jointly analyzing over 1000 melanoma samples. We identified 32 genes that exhibited statistically significant (FDR < 0.01) evidence of positive selection, 19 of which were not identified as such by any one of the five studies. Positive selection on mutations in 10 of the 32 genes was supported by co-occurring increases in gene copy number, or loss-of-heterozygosity. The 32 genes were collectively enriched for those involved in the MAPK signaling pathway, cell division, immune evasion, and subunits of the SWI/SNF complexes and MLL complexes. Moreover, mutations in one X-linked gene were exclusive to male patients. To further understand the consequences mutations in the 32 genes, we studied their interplay with the melanoma transcriptome. Using non-negative matrix factorization, we untangled melanoma cell intrinsic and non-melanoma cell gene expression, identifying three intrinsic transcriptional signatures: one was characterized by overexpression of genes involved in oxidative phosphorylation, another by upregulation genes targeted by SMARCA2, a subunit of the SWI/SNF chromatin remodeling complexes, and an invasive signature associated with low expression of the MITF transcription factor. The relationships between these transcriptional signatures and mutations in a subset of the 32 genes demonstrated selective constraints on activating mutations in the KIT protooncogene and highlighted the importance of protein kinase A (PKA) signalling in melanoma. In summary, we present the largest mutation significance analysis in melanoma to clarify the landscape of genetic drivers of this disease.

QUALITY OF LIFE INDIRECT TREATMENT COMPARISONS OF NIVOLUMAB VERSUS PLACEBO AS ADJUVANT TREATMENT FOR MELANOMA
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The Phase III randomized controlled trial (RCT) CheckMate 238 (CM238) compared nivolumab versus ipilimumab as an adjuvant treatment of resected stage IIIB-IV melanoma (AJCC 7th edition). A Phase III RCT CA184-029 (029) compared ipilimumab versus placebo as an adjuvant treatment of resected stage IIIA-IIIC melanoma (AJCC 6th edition). Both trials assessed quality of life (QoL) using the EORTC QLQ-C30 questionnaire; analyses presented here compare the QoL findings of nivolumab versus placebo.

Bucher indirect treatment comparisons (ITCs) of nivolumab versus placebo were constructed using data from CM238 and 029 for 15 outcomes derived from the QLQ-C30 responses. Unadjusted hazard ratios (HRs) within trial were derived for time to deterioration (decrement of 10) for each outcome separately. ITCs were performed for each outcome using the intent-to-treat (ITT) population from each trial, and separately for the subgroup of stage IIIB/IIIC patients from each trial.

Nine of the 15 ITT ITCs resulted in HRs favouring nivolumab (physical functioning, role functioning, emotional functioning, cognitive

functioning, pain, insomnia, constipation, diarrhoea, financial difficulties), though the confidence intervals (CIs) include 1. Of the 6 ITT ITCs that favoured placebo (global health status, social functioning, fatigue, nausea/vomiting, dyspnoea, appetite loss), only the CI for dyspnoea does not include 1. The ITC results for the stage IIIB/IIIC subgroup matched the ITT results closely, with 9 of 15 HRs favouring nivolumab. ITT HRs ranged from 0.87 to 1.37, and subgroup HRs from 0.76 to 1.42.

Based on ITCs, nivolumab demonstrated similar QoL to that of placebo. These QoL ITC results should be reviewed in parallel to efficacy and safety outcomes to fully evaluate the risk:benefit profile of nivolumab for the adjuvant treatment of resected melanoma with lymph-node involvement.

EVALUATING THE RELATIVE EFFICACY OF NIVOLUMAB VERSUS PLACEBO AS ADJUVANT TREATMENT FOR MELANOMA USING MULTIPLE METHODS OF INDIRECT TREATMENT COMPARISON

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The Phase III randomized controlled trial (RCT) CheckMate 238 (CM238) demonstrated the safety and efficacy of nivolumab as an adjuvant treatment for melanoma in patients with Stage IIIB/C or IV disease (AJCC 7th ed.) versus ipilimumab. As the CM238 study used an active comparator, there is currently no data directly comparing the efficacy of nivolumab to observation (placebo) in this indication.

Indirect treatment comparisons (ITCs) of nivolumab versus placebo were constructed using data from CM238 and CA184-029. CA184-029 is a phase III RCT comparing ipilimumab with placebo in patients with resected stage IIIA-IIIC melanoma (AJCC 6th ed.). Unadjusted and covariate adjusted (including disease stage, age and sex) ITCs were performed using the Bucher method and patient level data for the outcomes of recurrence-free survival (RFS) and distant metastases-free survival (DMFS). ITCs based on subgroup analyses restricting each RCT to stage IIIb/IIIc patients were also performed. Results from a broader network meta-analysis were also considered for comparison.
In all ITC analyses, nivolumab performed significantly better than placebo with all estimated confidence intervals (CIs) for the RFS and DMFS hazard ratios (HRs) excluding 1. For RFS, the range of point estimates for HRs (nivolumab vs placebo) were 0.50 to 0.53. For DMFS, the range of point estimates for HRs (nivolumab vs placebo) were 0.57 to 0.62. The stage IIIb/IIIc subgroup analyses yielded HRs that were very similar to the ITT analyses; ranging from 0.51 to 0.52 for RFS, and 0.61 for DMFS.

Based on ITCs, nivolumab was associated with significantly greater RFS and DMFS than was placebo. Multiple methodological approaches were explored, and a high level of consistency has been found in both the magnitude of effect and interpretation of the results.

Sexual dimorphism in response to $BRAF^{mut}$ melanoma targeted therapy.

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We have made significant progress in the treatment of $BRAF$-mutant melanoma with the use of targeted therapy (TT), and these agents are now being used to treat earlier stage disease. We recently reported results of a phase II trial investigating neoadjuvant TT in patients with high-risk resectable melanoma, showing high RECIST and pathologic complete response (pCR) rates (85% and 58%, respectively). Importantly, pCR was associated with a high likelihood of long-term benefit. Interestingly, in this cohort we noted that a significant proportion of patients achieving a pCR were female (5/7, 71%). This trend was maintained in a retrospective cohort of patients receiving similar TT off-protocol (pCR at surgery in 8/21 patients, all female), and continues in ongoing accruals per-protocol to date (pCR at surgery in 4/9 patients, all female).
Using a syngeneic $Braf^{V600E}/Pten^{-/-}$ murine melanoma model, we demonstrated enhanced tumor control by combination TT in female animals, consistent with our clinical observation. To explore the mechanism by which gender might be influencing therapeutic response, we first examined whole exome sequencing and transcriptomic data from available pre-treatment patient tumors but found no clear differences predicting pCR or a gender effect. We next used a panel of patient-derived $BRAF^{V600mut}$ melanoma cell lines and identified a markedly dimorphic expression of GPER1, being virtually absent in male melanoma cells. GPER1 signaling, preferentially activated in female models, appears to confer sensitivity to TT through a mechanism that includes regulation of MYC. Together, these data suggest an underlying interaction between a sex-specific environmental factor and sex-based differences in signal pathway utilization in melanoma cells. Further studies are underway to fully characterize the mechanisms underlying this phenomenon and potential strategies to enhance pathologic responses.

TOP1 is associated with molecular signatures in melanoma progression and resistance

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The discovery of new targets have been revolutionized the ability to predict progression and monitor therapy in melanoma, however, resistance to MAPK inhibition remains a key issue. Topoisomerase 1 (TOP1) has long been considered a target for the treatment of human tumors and TOP1 inhibitors have been widely used in the clinic. However whether TOP1 might represent an important therapeutic target in melanoma remains unexplored. Here we show that TOP1 expression increases during melanoma progression and is reduced in therapy-resistant cells, especially those bearing activating BRAF mutations. By contrast no correlations were observed for other topisomerase isoforms (TOP1mt, TOP2A, TOP2B, TOP3A and TOP3B). Notably in the TCGA melanoma cohort TOP1 expression correlated with genes as BRAF, SOX10, AXL, JARID1B and MSX1 that are important molecular signatures in melanoma progression and resistance. In addition, in melanoma cultures TOP1 expression showed to be associated with a proliferative phenotype (SOX10, MITF) but was strongly anti-correlated with AXL, a hallmark of de-differentiation and drug resistance. A TOP1 inhibitor (Topotecan) with IC50 values between 0.2 and 1.5 µM prevented religation of single strand breaks generated by during replication and associated with acquisition of resistance in BRAF-melanomas. These results suggest that TOP1 plays a key role during melanoma progression and adaptive resistance to BRAF/MEK.

Premature senescence in human melanocytes after exposure to solar UVR: An exosome and UV-miRNA connection

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Sustained exposure to ultraviolet radiation (UVR) is a well-established factor in converting melanocytes into melanoma. An important question is whether daily exposure to UVR affects these cells by inducing them prematurely into a senescent-like state, and thereby making them more susceptible to transformation upon further exposure to UVR. We therefore developed an *in vitro* model to examine whether repeated doses of UVR (UVA + UVB) could hasten human melanocytes into senescence and whether this process is guided by the release of exosomes carrying certain miRNAs as a part of this process. Thus, quiescent melanocytes were exposed twice over a 24 hour interval with sub-lethal doses of solar simulated UVR (ssUVR) and then returned to complete and exosome-depleted media for 40 hours. We found that ssUVR could indeed induce senescent-like properties in melanocytes, as judged by known senescence markers and the repression of certain DNA repair genes. Moreover, these cells secreted extracellular vesicles having characteristic features identical to those of exosomes. We then used microRNA arrays and qRT-PCR to identify the miRNAs that were carried by these vesicles. The results of this analysis were striking in that we found that a majority of the incorporated UV-miRNAs could target, and with statistical confidence, genes comprising a senescence core signature and genes encoding well known senescence-associated secretory factors (e.g., cytokines, chemokines) and apoptotic proteins. Thus, our data suggests that the removal of specific miRNAs by these exosomes likely helps to create a premature senescence in melanocytes after repeated exposure to solar UVR; and further cumulative exposure to UVR while in this senescent state (along with a decreased DNA repair capacity) could then lead to irreversible DNA damage in melanocytes, with eventual possible melanoma formation.

Recurrent pneumonitis in melanoma patients treated with Immune checkpoint inhibitors (ICI)

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Background: Alongside impressive anti-tumor responses, ICI gave rise to new novel immune related adverse events (irAE). Pneumonitis is an irAE that requires a careful management, yet no consensus exists regarding steroid treatment duration or drug rechallenge. Our study describes the clinical and radiological course of melanoma patients diagnosed with pneumonitis, that has recurred due to rechallenge attempt or despite complete treatment discontinuation.
Methods: Study population was composed of metastatic melanoma patients who were treated with anti-PD-1 as monotherapy or in combination with anti-CTLA-4, and were diagnosed with pneumonitis. For recurrent cases after clinical and radiological resolution, we examined the differences from cases with no recurrence.
Results: Nineteen out of 386 (4.8%) patients treated with ICI were diagnosed with pneumonitis. Median age was 66 years and 53% were male. Compared to single agent Nivolumab, patients treated with Ipilimumab-Nivolumab combination presented with an earlier onset (27.5w vs. 10.3w respectively, p=0.015), and presented higher grades of severity. After complete resolution, rechallenge was attempted in 7 patients; three of them had recurrent pneumonitis. Three other patients experienced recurrent pneumonitis despite complete discontinuation of the drug. The later were compared to those who did not experience recurrence: their first event of pneumonitis seemed to occur earlier (median 12.4w vs. 26.4 weeks, p=0.08) and steroid treatment duration at first event was shorter (5.1 vs 10 weeks, p=0.184). Recurrent cases were generally more severe than the first event.

Conclusion: Unprovoked recurrent pneumonitis is a new, poorly reported entity. The results of this study suggest that these cases present with an earlier onset and that they may be predicted, thus enabling a closer monitoring and a longer course of steroid treatment.

DESCRIBE III: A Retrospective Analysis of Dabrafenib (D) and/or Dabrafenib Plus Trametinib (D+T) in Patients With Metastatic Melanoma (MM) Participating in the Individual Patient Program (IPP)

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With approved targeted and immunotherapy regimens, optimizing treatment decisions for pts with MM remains an important goal. Long-term follow-up in clinical trials of D+T demonstrated durable benefit in pts with *BRAF* V600 mutation–positive MM (3-y overall survival [OS] rate of 45% [Robert et al, ESMO 2016 (abstract LBA40)]), but there is a paucity of real-world data. The DESCRIBE I (D monotherapy) and DESCRIBE II (D+T) studies examined real-world outcomes, which were consistent with those of previous clinical trials; however, longer observation time is needed. DESCRIBE III is a global, observational, retrospective chart review study evaluating baseline characteristics, treatment patterns, and efficacy and safety outcomes in pts with various durations of benefit: long-term (LT; on therapy ≥12 mo), intermediate duration (ID; on therapy ≥6 to <12 mo), and short-term (ST; on therapy <6 mo). Pts aged ≥18 y with *BRAF* V600–mutated MM or unresectable melanoma who received ≥1 dose of D and/or D+T in the IPP were eligible. As of June 2018, 38, 23, and 23 pts in the LT, ID, and ST benefit groups, respectively, were analyzed. Median age across all groups (N=84) was 59.5 y, and 25 of 84 pts (30%) were continuing therapy at the data cutoff. Overall, 62% of pts received ≥1 prior antineoplastic therapy (LT, 47%; ID, 70%; ST, 78%). Efficacy analyses are ongoing and will include overall response (CR+PR) and clinical benefit (CR+PR+SD for >24 wk) rates, median progression-free survival, and median OS in the 3 benefit groups. Safety analyses will include AEs of special interest and AEs leading to discontinuation. These results will further increase our understanding of the relationship between baseline characteristics, duration of clinical benefit, and safety outcomes in a real-world population.

Results from a Phase I dose escalation trial (TACTI-mel) with the soluble LAG-3 protein (IMP321, eftilagimod alpha) together with pembrolizumab in unresectable or metastatic melanoma

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IMP321 is a recombinant soluble LAG-3Ig fusion protein binding to MHC class II molecules and mediating antigen presenting cell (APC) activation followed by CD8 T-cell activation, which may lead to stronger anti-tumor responses. Combining an APC activator with an immune checkpoint inhibitor (ICI) aims to increase efficacy without additional toxicity. We report results of the dose escalation part A of a phase I trial (NCT02676869) with pembrolizumab and IMP321. In this prospective study, melanoma patients (pts) on pembrolizumab (2 mg/kg i.v.) with progressive disease (irPD), stable disease (irSD) or partial response (irPR) acc. to irRC after 3 cycles received 1 mg (n=6), 6 mg (n=6) or 30 mg (n=6) s.c. injections of IMP321 (every 2 weeks for 6 months) from cycle 5 of pembrolizumab onwards. Eighteen pts (17 male, 1 female) with a median age of 66 years (range 48-85) were enrolled. Fifteen (83 %) and seven (39 %) pts had stage M1C disease and elevated LDH, respectively. Seven (39 %) pts completed the 6 months combination treatment. The most common AEs were fatigue (44 %), rash (33 %), diarrhea (28 %), nausea (28 %), arthralgia (17 %) and colitis (11 %). One patient experienced intracranial hemorrhage grade 4, one had a colitis grade 4, both not related to IMP321 or pembrolizumab. No dose limiting toxicity has been reported. Objective response rate taking as baseline target lesions at cycle 5 of pembrolizumab was 33 % (irRC) including one patient with a confirmed irCR after irPD on pembrolizumab monotherapy. Up to 30 mg IMP321 in combination with pembrolizumab are safe and well tolerated. The responses observed in pts with suboptimal response to pembrolizumab alone may point to a benefit of adding a systemic APC activator to an ICI.

Identification of signalling pathways, genes and miRNAs dysregulated in high metastatic risk uveal melanoma

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Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, characterized in ~50% of patients by a propensity to metastasize to the liver, which is then fatal. Patients can be stratified into low (LR) or high (HR) metastatic risk groups. Using transcriptomics, genes and their effectors that contribute to these metastatic phenotypes can be identified. This information will be essential in the search for therapeutic targets.

Total RNA was extracted from tumour tissue of 10 LR and 10 HR UM cases and hybridised to human Affymetrix GeneChip microarrays. Differentially expressed genes and miRNAs were identified using bioinformatic pipelines, and Ingenuity Pathway Analysis (IPA) was employed to identify relevant molecules and pathways. Validation of highly differentially expressed genes/miRNAs was undertaken by qPCR.

Bioinformatic analysis of the transcriptome identified 895 differentially expressed genes (fold change >2, p<0.05); 372 genes were upregulated and 523 downregulated in HR *c.f* LR group. Also 149 miRNAs were differentially expressed with fold change >2; 56 miRNAs upregulated and 93 downregulated in HR *c.f* LR group. Genes and miRNAs previously implicated in UM were identified: *HTR2B*, *ENPP2*, *hsa-miR-181* and *hsa-miR-509*. Using qPCR, expression of genes/miRNAs, *HTR2B*, *PDE3A*, *hsa-miR-509-3p*, *hsa-miR-1296* were consistent with microarray data. IPA identified cancer as the main biological function and highly-upregulated canonical pathways in HR UM included EIF4 signalling; whilst EIF2 signalling is downregulated. UM cell lines were profiled and gene/miRNA expression varied dependent on cell line, providing key information regarding appropriate UM cell line models for functional analyses.

Transcriptomic analysis has identified pathways, genes and miRNAs dysregulated in HR UM, which require further functional validation as novel therapeutic targets.

IN VIVO SCREENING OF FUNCTIONAL NOVEL ONCOGENIC ENHANCERS/SUPPRESSORS IN A ZEBRAFISH MELANOMA MODEL
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A model for melanoma progression has been developed in zebrafish based on most frequently mutated human gene in melanoma: BRAF. In a zebrafish melanoma model over-expression of histone methylase SETDB1 accelerates the onset of melanoma development. Most recently, Busch et al demonstrated that loss of chromatin modifier Kdm2aa causes BRAF^{V600E} independent spontaneous melanoma in zebrafish. These observations are in line with the RNA analysis done previously in our lab on 15 congenital naevi versus 13 primary melanomas revealing potentially interesting genes whose functional consequences are only partially known. To functionally test candidate genes for the ability to accelerate melanoma transgenic zebrafish that over express candidate genes such as PIK3CA^{H1047R}, YAP-1, STK11, NRAS^{Q61K} and BRAF^{V600E} on a Tp53 mutant background was observed. The target genes were tested under 2 different promoters-MITF and SOX10. In addition to this , epigenetic genes MAPK14 and TET2 will be tested for melanoma enhancer/suppressor in zebrafish melanoma of BRAF and NRAS mutant origin. Multisite gateway technology in combination with HiFi DNA assembly was used to create the expression constructs which were injected in 1 cell stage Nacre mutant embryos to create stable transgenic lines. The tumor incidence rate in Tg(mitf:BRAF^{V600E})p53(lf)mitf(lf) was 21. 4% compared to 100% in Tg(mitf:NRAS^{Q61K})p53(lf)mitf(lf). The Tg(mitf:STK11)p53(lf)mitf(lf) did not show any phenotypic effect yet. The Tg(SOX10:BRAF^{V600E})p53(lf)mitf(lf) have a poor survival rate and Tg(SOX10:NRAS^{Q61K})p53(lf)mitf(lf) are embryonic lethal. Further, the effect of MAPK14 andTET2 in melanoma progression will be tested in Tg(mitf:BRAF^{V600E}/NRAS^{Q61K})p53(lf)mitf(lf) by over-expressing them in melanocytes.

Preliminary Results from a Microbiome-based Phase I Clinical Trial - Fecal Microbiota Transplantation in Metastatic Melanoma Patients Who Failed Immunotherapy (NCT03353402)

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Recent reports have demonstrated a clear correlation between gut microbiome composition and clinical response to immunotherapy. Fecal microbiota transplantation (FMT) experiments in murine models showed that feces from patients who responded to immunotherapy enhanced immunotherapy efficacy. Here, we report a clinical trial aimed at using FMT in humans in order to induce a clinical response to anti-PD-1 therapy. FMT donors were metastatic melanoma patients with a durable complete response to anti-PD-1 therapy. FMT recipients were patients who failed all standard lines of treatment, including other Phase I trials. FMT was delivered via both colonoscopy and stool capsules. Collected pre and post-FMT samples included: stools for 16S rRNA gene sequence and metagenomic analyses; serum for cytokines measurements; peripheral blood mononuclear cells for RNA sequencing; gut and tumor biopsies for histological analyses and RNA sequencing. To date, two patients have undergone FMT as monotherapy. Additional patients will undergo FMT and anti-PD-1 therapy combination in the coming weeks. No severe adverse events related to FMT were observed in these patients. Matched pre and post-FMT gut biopsies of the first patient demonstrated a significant increase in monocytic proliferation and a slight increase in CD4+ lymphocytic infiltration in the lamina propria. Imaging studies conducted two months post-FMT for the first patient demonstrated progressive disease, however, tumor progression rate was lower when compared to previous imaging. In conclusion, FMT in the setting of heavily pre-treated metastatic melanoma patients appears to be safe and might induce an immune response to the implanted microbiome. Combining FMT and anti-PD-1 therapy will enable us to assess the effect of the FMT on clinical response to therapy.

Variability in the Treatment and Prognosis of Nodal Disease in Melanoma at the Extremes of Age: A NCDB Analysis

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Background: As clinicopathologic characteristics of melanoma vary by age, we sought to evaluate the application of adult treatment algorithms to pediatric and elderly melanoma patients, specifically the application of nodal surgery and systemic therapy and the impact of these therapies on overall survival (OS).

Methods: We performed a National Cancer Database analyses of melanoma patients from 2004-2015. Patients were categorized by age (1-10 (pediatric), 11-25 (adolescent and young adult (AYA)), 26-40, 41-64, 65-79 (older adult), and ≥80 years old (elderly)). Non-parametric, log-rank and Cox proportional hazards regression analyses were performed to compare clinicopathologic characteristics, treatment, and OS. Due to the treatment implications for positive nodal disease, subset analyses were performed on patients with stage III disease.

Results: Of the 257,338 patients, 0.1% were 1-10, 2.8% were 11-25, 12.4% were 26-40, 47.7% were 41-64, 27.7% were 65-79, and 9.3% were ≥80 years old, with significant demographic, clinicopathologic, and treatment differences between age cohorts. Pediatric and AYA was associated with improved OS compared to stage-matched adults with IIB-IIIC disease (p<0.05). Among stage III disease, immunotherapy was more common among pediatric and AYA cohorts and least common among the older adults and the elderly (p<0.0001). However, immunotherapy was associated with improved OS among older adults and the elderly (HR 0.67, 95%CI 0.59-0.75, p<0.0001), but not pediatric and AYA patients (p>0.05).

Conclusions: These significant clinicopathologic, treatment, and prognostic differences in the very young and old melanoma patient illustrate the heterogeneity of melanoma across age groups and suggest a need for tailored treatment approaches in pediatric and elderly patients.

Antibody profiling to predict responses to immunotherapy in melanoma

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Advances in melanoma treatment include targeting key immune checkpoints, such as programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). While highly effective in some, the majority of patients are not responding and treatment related adverse events (irAEs) are common and often severe. Therefore, the ability to predict treatment outcomes is of utmost value. Treatment success is often correlated with an immunological active tumour, but the majority of the commonly-used immune monitoring techniques to investigate tumour-immune engagement rely on access to patient tissue via invasive surgeries or biopsies. As such, there is a clear preference for a blood-based means of assessing immune engagement, with the intent of obtaining a more accurate representation of the majority of all tumours. Tumour antigens, including tumour-specific antigens (TSAs) and tumour-associated antigens (TAAs), can enable malignant cell recognition by the immune system and subsequently lead to the production of specific

antibodies. We hypothesized that these antibodies can be informative markers of immune engagement of tumours. Here we used a novel protein array which represents a high-throughput, sensitive tool capable of profiling antibody repertoires of cancer patients using only 1μl of serum or plasma. Importantly, healthy individuals show no detectable cancer-specific antibody titers. Preliminary data (unpublished) using the array on a small subset of patients (n=15) undergoing ICB treatment with pembrolizumab (PD-1 blockade) was generated. Pre-treatment data shows a trend towards separation of clinical responders from non-responders using number of antigen specificities and mean antibody intensity. Furthermore, immunotherapy treated patient antibody profiles may be useful to predict irAEs ahead of clinical evidence.

Ipilimumab-Nivolumab combination therapy in metastatic melanoma patients who have progressed on previous anti PD-1 therapy

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Introduction
Immunotherapy with anti PD-1 agents and combination of anti PD-1 and anti CTLA-4 has changed the landscape of current treatment for metastatic melanoma. Effective advanced line therapy after progression on anti PD-1 agents and BRAF+MEK inhibition is lacking, relying mostly on treatment with Ipilimumab, with discouraging results. We report a case series of patients who have progressed on previous anti PD-1 therapy who were subsequently treated with Ipilimumab-Nivolumab combination.

Methods
Patients records were analyzed retrospectively; 22 patients were treated with Ipilimumab+Nivolumab after progression on anti PD-1 therapy.
Baseline characteristics, prognostic factors, response rate, duration of response and toxicity were analyzed.

Results
14 patients were treated with the combination as 2nd line (BRAF wild-type), 8 patients treated as 3rd line (BRAF mutant). Median number of treatment cycles was 4. 31.8% of patients achieved an objective response, including 3 complete responses and 4 major partial responses. Median PFS for all patients was 4 months. Median PFS for responding patients was 14 months with response still ongoing in 57% of responding patients at a median follow-up time of 14 months (range 3m-23m). No correlation was found between response to ipilimumab-nivolumab and line of therapy (p=0.67) or previous response to anti PD-1 therapy (p=0.85). Toxicity was on par with known data with 54.5% grade 2 or above immune adverse events(irAE). A trend was noted between appearance of G2-4 irAE and response to therapy (p=0.13).

Conclusion
Ipilimumab and Nivolumab combination therapy yields significant responses in metastatic melanoma patients who progressed on previous anti PD-1 therapy, both as 2nd and as 3rd line of therapy. Further, prospective trials are warranted.

Immunotherapy comes of age in octagenarian and nonagenarian metastatic melanoma patients

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Introduction
Immunotherapy with anti PD-1 agents has proven efficacy in metastatic melanoma across all age groups. Recent data hint at better response to therapy for patients over age 65. Elderly patients in their 80's and 90's pose a clinical challenge as they are less tolerable to adverse events and suffer from multiple co-morbidities. Here we describe a cohort of 146 patients ≥65 years old patients and analyze the efficacy of anti PD-1 therapy in patients ages 80-100 compared to patients ages 65-80.
Methods
Records of metastatic melanoma patients of ages 60-100 treated with anti-PD-1 agents were collected retrospectively. Baseline parameters, response rate (ORR) and best response(BR) were analyzed. Logistic regression and chi^2 test were employed for statistical analysis.
Results
510 patients were treated with anti PD-1 agents between 2014-2018. 146 patients were >65 years, 82 (56%) were 65-79 years (median 71), 64(44%) were ages 80-100(median 83, range 80-97). Baseline parameters (ECOG PS, LDH, BRAF, line of therapy, M stage) were comparable between the groups with the exception of a trend for more advanced line in the 65-79 age group (p=0.062) and significantly worse PS in patients over 80 (p=0.002).
123 patients were evaluable for analysis of response (76 ages 65-79, 47 ages 80-100).

ORR was significantly higher for the older population with 45/76 patients (59%) ages 65-79 and 37/47 patients (79%) ages 80-100 responding to therapy (p=0.026). Significantly more patients in age group 80-100 vs ages 65-79 achieved a complete response(CR) (55% vs 20%, p=0.000). Toxicity for patients ages 80-100 was on par with known data at 22% G2-4 adverse events.
Conclusion
Elderly patients show enhanced response to immunotherapy with anti PD-1 agents.
Increasing age within the elderly patients group may predict an even better response to therapy with higher ORR and CR rate in patients of very old age.

Responders to anti-PD-1 therapy: long-term outcomes and responses to retreatment

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Durable responses to anti-PD-1 therapy have prompted many clinicians to discontinue treatment in patients (pts) with excellent responses. However, relapses after discontinuation are poorly understood and little is known about retreatment with a second course of anti-PD-1. We conducted a retrospective analysis of pts with advanced, unresectable melanoma treated since 2009 at MSKCC with ≥1 dose of anti-PD-1 monotherapy followed for ≥3 months, excluding uveal melanoma (n=277). Complete response (CR) was defined by radiographic absence of disease or a negative biopsy of residual tissue. Overall survival (OS) was calculated from anti-PD-1 start.

Median follow-up was 36.5 months (mos) for survivors. 3-year OS was 47.6%. For the 65 pts (23%) who achieved CR, therapy was discontinued due to CR (n=50), toxicity (n=11), or other reasons such as protocol completion (n=4). 55 of the CR patients (85%) remain disease-free with a median CR duration of 25 mos (range 3-61) and median duration off therapy of 21 mos. 3-year relapse-free survival was 88%. 10 CR pts later had progressive disease (PD): 1 with a new brain lesion, 1 at a prior disease site, and 8 with new extracranial lesions.

27 of the 277 pts received a second course of anti-PD-1 for PD after a median of 12 mos (range 3-28), leading to 1 CR (pt had a partial response [PR] after first course), 2 PRs, 7 (26%) stable disease (SD), and 17 (63%) PD. 4 pts with initial CRs were retreated; 3 had SD and 1 had PD. 7 pts with initial PD were retreated; 2 had PRs and 5 had PD.

85% of pts who achieve CR remained disease-free even after discontinuing anti-PD-1. In this large dataset with some of the longest follow-up reported, it is clear that late recurrences occur. Pts retreated with anti-PD-1 had a low response rate, even if they achieved excellent responses initially. We are currently performing a multivariate analysis for complete responders.

Immunotherapy for vulvo-vaginal melanoma

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Introduction: Series describing the outcome of immunotherapy for vulvo-vaginal melanoma are lacking. Herein we describe a single site experience with patients treated for locally advanced and metastatic vulvo-vaginal melanoma.
Patients: Patients who were treated since 2013 are included in this case control analysis.
Results: 23 patients were treated for vulvo-vaginal melanoma. Median age was 65 years (range 21 to 79). 16 with vulvar melanoma and 8 with vaginal melanoma. 17 were treated with systemic therapy for metastatic or locoregional non resectable disease (7/10: vaginal/vulvar). 5/2/8 and 2 patients were treated for for stage IIIc/ IV M1a/ M1B and M1c disease respectively. All received anti PD1 antibodies (pembrolizumab or nivolumab) monotherapy, of them as first line in 11 patients. 5 received Ipilimumab, 4 received combined Ipilimumab and Nivolumab, 3 received Interleukin 2 based therapy and 4 received chemotherapy. One patient achived PR to PD1 inhibitor (6%), and 7 achived SD (41%) of whom 2 experienced pseudoprogression followed by prolonged stability (27 and 39 months). 9 patients had PD (53%). Median PFS was 8 months. Grade 3/4 toxicity occurred in 3 patients (arthritis, colitis, DM). 1 patients achieved CR to combined ipilimumab and nivolumab post pembrolizumab failure. One achieved CR twice in response to ipilimumab and one achieved CR with chemotherapy given following failure of 2 immunotherapy lines. One patients only had BRAF mutation. Median overall survival of all immunotherapy treated patients was 66 months (range 1.5 to 120) with no significant difference between patients with IIIc/IVM1A to M1B/M1c disease (66 vs 14 p=0.305).Vaginal melanoma correlated with worse outcome compared to vulvar melanoma (12 vs 66 mo, p=0.006, HR-0.19 95%CI 0.04-0.8).
Conclusion: Immune checkpoint inhibitors may induce prolonged benefit and durable survival in patients with locally advanced and metastatic vulvovaginal melanoma.

PD-1 inhibitor-induced autoimmune lipodystrophy and recurring sarcoid reaction in patient with metastatic melanoma

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PD-1 inhibitors activate T-cells causing an attack on cancer cells. However, they may also attack normal cells. Here, we report loss of body fat following PD-1 inhibition. The mechanism is most likely an autoimmune destruction of fat cells.

A 51-year old man with melanoma in lymph nodes and spleen started treatment with PD1-inhibitor nivolumab in September 2016. After 1 year, he developed a symptomatic sarcoid reaction with nodules in both lungs. Therefore, nivolumab was discontinued and he started 100 mg prednisone daily. He improved and prednisone was tapered until discontinued in January 2018. At that time, PET/CT-scan showed complete response but also loss of subcutaneous and visceral fat while simultaneously showing fat accumulation in the liver. He experienced a weight loss of 6 kg and pain when walking without shoes due to fat loss in the soles of his feet. In April 2018, CT-scan and biopsies revealed reactivation of sarcoidosis in lungs and lymph nodes. Strikingly, the patient now had complete loss of body fat. Triglycerides were high and he had developed insulin resistance with hypertension and microalbuminuria, all fitting the picture of lipodystrophy. Lipodystrophy is a rare syndrome associated with autoimmunity but not previously reported following PD1-inhibition (PubMed, Embase, VigiBase). The patient has been started on 60 mg prednisone and angiotensin II antagonist and referred to a diabetologist and a lipidologist. A new PET-CT one month after restarting cortisone showed a significant improvement of the sarcoid reaction in the lungs and lymph nodes but still no visible change in his lipodystrophy. He feels sufficiently well to be working full time.

In conclusion, we describe a case of autoimmune lipodystrophy following nivolumab treatment.
Treatment of this secondary metabolic syndrome is important to avoid long term morbidity.

Spectroscopic Biofluid Diagnosis, Monitoring and Therapeutic Profiling of Melanoma Patients
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Blood based biomarkers of cancer are attractive as a means of assessing and monitoring disease. They are less invasive and more easily accessible than traditional radiological or tissue based approaches. Blood based biomarkers could be used to monitor treatment progression and/or to monitor for early identification of disease relapse. Recent research has focused on circulating free DNA; here we propose a novel serum based approach utilising attenuated total reflectance - Fourier transform infrared (ATR-FTIR) spectroscopy as a blood based biomarker approach for melanoma.
Biomedical spectroscopy has been used to diagnose a wide range of pathologies (critically at an early stage), including brain cancer, endometrial cancer and prostate cancer with high sensitivity and specificity. However, no study to date has investigated serum spectroscopy in melanoma patients.
This research project established a longitudinal sample biobank consisting of 297 melanoma patient serum samples from 110 patients, acquired over up to 8 repeat clinical appointments. This resource has been fully analysed using recently developed novel clinical ATR-FTIR technology.
Through a process determined to be health economic for the analysis of brain tumours, 3 microlitres of serum was analysed, providing results within 10 minutes to demonstrate for the first time the ability of ATR-FTIR to:
1) Determine the BRAF status of patients with metastatic melanoma currently to levels of 75% sensitivity and 77% specificity
2) Follow specific patients through their treatment and have a proposed metastatic spectroscopic profile
Future work will concentrate on improving sensitivity and specificity of these methods and mapping patients on treatment to monitor spectroscopy profiles. These promising results could lead to the development of novel blood based biomarkers for patients with melanoma.

High-dimensional "in situ" single cell-analysis of tumour infiltrating lymphocytes (TILs) in primary melanoma using Multiplex Immunostaining on tissue sections.
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A "high resolution" investigation of the tumor microenvironment has been urged in recent years by the introduction of immunotherapy in clinical practice. In particular, analysis of the heterogeneity of the immune infiltrate, its spatial distribution as well as the function of each component within the infiltrate, has become mandatory. We have used a technology with single cell resolution (Bolognesi et al 2017) in order to study the primary melanoma microenvironment "in situ" (i.e. on tissue sections) integrating its morphological and functional features. We characterized the immune landscape at the single cell-level in 29 primary melanomas based on a panel of 40 immune markers applied on one single tissue microarray section using a high-dimensional immunophenotypic characterization. In particular, we studied the immune microenvironmental differences between melanomas with brisk and non-brisk infiltrates and with early and late regression both in terms of quantity as well as social organization of the inflammatory cells, using neighborhood analysis. The data were validated by RT-PCR expression and shotgun proteomic analysis. This approach allowed us a) to demonstrate

that brisk and non-brisk patterns do not differ significantly in the activation status of their TILs but can be further subdivided into three functional categories, i.e. predominantly active, transitional and predominantly exhausted; b) to investigate the most important inflammatory subpopulations involved in the process of TILs exhaustion; and c) to define the correlation between T-cell activation and spontaneous melanoma regression. This resulted in a "dynamic" picture of the "in situ" inflammatory microenvironment in melanoma that may be helpful in the search for predictive markers in patients undergoing immune checkpoint therapy.

HRQoL results for pembrolizumab versus placebo after complete resection of high-risk stage III melanoma from the EORTC 1325-MG/Keynote 054 trial: an international randomized double-blind phase 3 trial.

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Background:
The EORTC 1325-MG/Keynote 054 trial demonstrated prolonged recurrence-free survival with adjuvant pembrolizumab compared to placebo (hazard ratio = 0.57; P<0.001. Eggermont et al, NEJM, 2018). Incidence of adverse events grades 3 or higher related to treatment were higher in the pembrolizumab arm (14.7%) than the placebo arm (3.4%). Here we report the health-related quality of life (HRQoL) results.

Methods:
A total of 1019 patients with histologically confirmed, cutaneous melanoma metastatic to a lymph node, classified as stage IIIA, IIIB or IIIC were randomized after complete resection to receive 200 mg pembrolizumab (514 patients) or placebo (505 patients). Treatment was administered every 3 weeks for 1 year or until disease recurrence or unacceptable toxicity.
All enrolled patients were required to complete a HRQoL questionnaire at baseline and every 12 weeks (during 2 years after randomization). The primary HRQoL outcome was global health/QoL (GHQ) as measured by the EORTC QLQ-C30. All other scales from this questionnaire were secondary.

Results:
HRQoL compliance was >90% at baseline, >70% during the first year and >60% thereafter for both arms. Data attrition limited the analyses to week 84 (19 months).
Baseline GHQ scores were similar between arms at 77 points and remained stable over time. The average GHQ score was 2.2 points (95% CI: 4.3-0.2), 1.1 points (95% CI: 3.2 - -0.9) and 2.2 points (95% CI: 4.8 - -0.4) lower in the pembrolizumab arm compared to placebo for the average overall, during and after treatment respectively. These differences are within 5 point clinical relevance threshold for the QLQ-C30.

Conclusion:
Pembrolizumab maintains HRQoL compared to placebo, when given as adjuvant therapy for patients with resected high risk stage III melanoma.

Diversity of BRAF fusion kinases in melanoma and their pre-clinical targeting

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Oncogenic BRAF fusions are among the most common kinase rearrangements. They are detected in an increasing number of neoplasms including a subset of melanocytic tumors. However, little is known about the features associated with BRAF fusions and their clinical management remains unresolved in part due to the scarcity of pre-clinical models for drug studies. In the literature 90 melanocytic tumors with BRAF fusions are reported, with a higher prevalence among patients of female gender and younger age. Contrary to some other tumor types with BRAF fusions, the spectrum of 5' partners is broad in melanocytic tumors. There is no clear effect of the nature of the fusion partner, its contribution of a dimerization domain, or the breakpoint location on clinical outcome. We surveyed a series of "wild type" melanoma cell lines and identified six presenting BRAF fusions as dominant driver oncogenes. We tested a broad range of RAF and MEK inhibitors and encountered unexpectedly heterogeneous responses that could be categorized upon the presence of specific features of the fusion kinases. Experiments carried-out in isogenic cell models confirmed that higher expression level of the fusion kinases resulting from the promoter strength of the fusion partner and/or copy number increase correlated with resistance. Moreover, presence of a dimerization domain in the fusion partner resulted in the paradoxical activation of the MAP-kinase signaling pathway and hyperproliferation in response to first- and second-generation RAF inhibitors. By contrast, the new generation αC-IN/DFG-OUT RAF inhibitors were highly effective in preventing paradoxical activation and inhibiting cell proliferation across all cell lines. Adding a MEK inhibitor to the new RAF inhibitors further increased the therapeutic efficacy in vitro and in vivo, opening new perspectives in the clinical management of tumors harboring BRAF fusions.

Facial-Aging Apps in Waiting Rooms: A New Opportunity for Melanoma Prevention?

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Around 90% of skin cancers are caused by UV exposure. A randomized controlled trial by Mahler et al. demonstrated increased sun-protective behavior at 4-5 and 12 months follow-up by the help of UV-photographs, in which the current damage of UV on the users own face is shown.

We took advantage of the waiting room of our HIV clinic and developed a kiosk mode for a free facial-aging app, in which a 3D-animated selfie is altered to predict future appearance, to develop an intervention that exposes the large majority of patients visiting a healthcare provider.

A tablet with the facial-aging app "Sunface" running was placed on a table in the middle of the waiting room and connected with a large monitor hanging on the opposite wall (Fig. 1). An interviewer was placed in the room to encourage all patients to try the app try the app if they had not done so themselves within 30 seconds of entering. All participants were asked to fill in an anonymous questionnaire.

272 patients were counted in a waiting room over 13 days (m=207/76.1%). 202/74.3% of the participants were encouraged to try the app after 30 seconds of entering; 26/9.6% tried it themselves within 30 seconds of entering; 16.2% patients waited outside the room or for less than 30 seconds. A total of 119 patients tried the app and agreed to fill out a questionnaire thereafter. 16.9% watched another patient without trying it themselves, so a total of 60.7% of the 272 patients were exposed to the intervention. 105/88.2% said the intervention motivated them to increase sun protection (m=74/89.2%; f=31/91.2 %), to avoid indoor tanning beds (m=73/87.9%; f=31/91.2%) and that they perceived the intervention as fun (m=83/98.8%; f=34/97.1%).

Facial-aging apps implemented in waiting rooms show the potential to provide a new enjoyable opportunity to motivate a large fraction of patients who visit a healthcare setting to increase their UV-protection.

Photoaging Mobile Apps in school-based Melanoma Prevention: Preliminary results from Germany and Brazil and perspectives for large scale implementation.

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Around 90% of melanomas are caused by UV-exposure. Tanning behavior is mostly initiated in early adolescence, often with the belief that it increases attractiveness; the problems related to malignant melanoma and other skin cancers are too far in the future to fathom.

We implemented a free photoaging mobile phone app (Sunface) in the school-setting in Germany and Brazil including more than 1,700 adolescents in total. We "mirrored" the students" altered 3-dimensional (3D) selfies reacting to touch on mobile phones or tablets via a projector in front of their whole grade. Prospective and cross-sectional data were collected via questionnaire.

We measured more than 60% agreement in both items that measured motivation to reduce UV exposure in both countries: For Germany, the perceived effect on motivation was increased in participants with Fitzpatrick skin types 1-2 in both tanning bed avoidance (n=74, 71.8% agreement in skin types 1-2 vs n=50, 53.8% agreement in skin types 3-6) and increased use of sun protection (n=70, 68.0% agreement in skin types 1-2 vs n=52, 55.3% agreement in skin types 3-6), and also positively correlated with higher age. In Brazil, the perceived effect on motivation was higher in female pupils in both tanning bed avoidance (n=198, 92.6% agreement in females vs n=123, 87.2% agreement in males) and increased use of sun protection (n=197, 92.1% agreement in females vs n=123, 87.2% agreement in males) and independent of age or skin type. Prospective results on behavior are still pending.

Preliminary results suggest that the intervention has a high probability of altering UV-protection behavior in adolescents. In addition, the concept of facial-aging interventions is transferable to high UV-index countries with a higher melanoma prevalence such as Brazil.

Expression of TAM receptors in melanoma of Korean patients

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Receptor tyrosine kinases (RTKs) are frequently ectopically expressed, overexpressed, or hyperactivated in tumor cells and are therefore attractive targets for cancer therapy. Overexpression of TAM (TYRO3, AXL, MER) family of RTKs has been observed in a spectrum of human cancers including melanoma and studies demonstrating that Axl and Mer contribute to mechanisms of cell survival, migration, invasion, metastasis, and chemosensitivity justify further investigation of Axl and Mer as novel therapeutic targets in cancer. This study was performed to investigate the expression of TAM receptors in melanoma of Korean patients. A total of 46 melanoma cases were analyzed for the TAM expression, consisting of 31 cases of acral lentiginous melanoma (ALM), 9 cases of superficial spreading melanoma (SSM) and 6 cases of nodular melanoma. Forty six primary tumors and eight metastatic tumors were immunohistochemically stained for TYRO3, AXL and MER. In addition, BRAF mutation status was also assessed by immunohistochemical analysis with BRAF[V600E].
Among 46 primary tumors, AXL, TYRO3 and MER expressions were observed in 97.1%, 87.0% and 37.0% of melanomas, respectively. In metastatic tumors, all 8 tumors expressed AXL and TYRO3 and 5 tumors (62.5%) showed MER expression. The expression of MER was more frequently found in SSM type and nodular type than in ALM type (55.6%, 50.0% vs 29.0%). MER expression was more frequently observed in melanoma thicker than 1mm than thin melanoma of T1 stage (41.1% vs 25.0%). In addition, Mer expression was more frequently detected in BRAF[V600E] expressing melanoma than BRAF[V600E] negative melanoma (53.3% vs 31.0%)
In this study, we observed that AXL and TYRO3 are commonly expressed in melanoma, while MER is expressed with advanced tumor in Korean patients. Therefore, TAM receptors can be considered as therapeutic targets in Korean melanoma patients, especially MER in advanced tumors.

Concurrent presence of intra-tumor CD20+ and CD8+ lymphocytes improves survival and response to immune checkpoint therapy in melanoma

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Checkpoint blockade therapies that reactivate effector T cells, such as anti-CTLA4 and anti-PD1, have provided long-term survival in a substantial fraction of patients. Currently, predictive biomarkers for therapy response include activity of intratumor effector cells and tumor mutational burden. Although the role of T cells in antitumor responses has been thoroughly studied, the role of other immunological cells remains insufficiently explored.
The presence of intra-tumoral immunological cell types was analyzed using immunohistochemistry in 177 metastatic melanoma tumors and, in addition, mutational and gene expression analysis were performed. Also, we obtained gene expression profiles of tumors from patients treated with anti-CTLA4.
We found that intra-tumoral CD8+ and CD20+ lymphocytes confer increased survival (P=0.016 and P=0.006, respectively). Overall, 33% of tumors had only CD8+ lymphocytes but no CD20+ lymphocytes, 25% had concurrent CD8+ and CD20+ lymphocytes, while 42% were absent for both markers. Tumors harboring both CD8+ and CD20+ lymphocytes had increased survival, as compared to tumors infiltrated with CD8+ lymphocytes only, which had intermediate survival, and tumors lacking both immune cells had the worst survival (P=0.006). We derived a gene expression signature consisting of predominantly B cell specific genes that characterized

CD20+/CD8+ melanoma tumors. This B cell signature was prognostic in independent primary and metastatic melanoma cohorts. The B cell signature predicted outcome in pre-treatment samples of anti-CTLA4 (n=39) and anti-PD1 (n=38) treated patients (*P*=0.025 and *P*=0.035, respectively).

Collectively, these results indicate that B cells have a key role in the immune microenvironment of melanoma tumors.

Outcomes of elderly patients (pt) with advanced melanoma treated with systemic treatments in the first-line setting: a multicentric analysis

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Elderly population is usually underrepresented in clinical trials so the actual efficacy of treatments in this subset of pt is not well known. We aim to assess the outcomes of this special population in first-line.

We retrospectively evaluated clinical characteristics, treatments, toxicity and outcomes of advanced melanoma elderly pt (≥65 yrs) treated with systemic therapy in first-line between OCT11 and NOV17 in 3 centers in Spain. We identified a total of 45 pt (60% M) with median age 72 yrs (65-87). 38% had BRAF V600mut, 27% M1a, 11% M1b, 58% M1c and 4% M1d. At treatment start, 78% pt had PS 0-1 (PS 2 the remaining) and 38% had high serum LDH. Most pt (91%) had monotherapy: 51% immunotherapy (2 pt ipilimumab, 21 pt nivolumab), 24% tyrosine-kinase inhibitor (TKI) (1 pt dabrafenib, 10 pt vemurafenib) and 16% chemotherapy (6 pt dacarbazine, 1 pt fotemustine). 4 pt received TKI combination (2 pt dabrafenib-trametinib, 2 pt vemurafenib-cobimetinib). Overall RR was 3 CR (7%) and 19 PR (42%). 7 pt had SD (15.6%). mOS was 17 months (m), with differences between PS 0-1 vs PS 2 (18.9 m vs 4.2 m, p=0.018), between normal basal LDH vs high (26.5 m vs 4.2 m, p=0.001). mPFS was 8.2 m, with differences between PS 0-1 vs PS 2 (9.2 m vs 1.6 m, p=0.047) and between normal basal LDH vs high (10 m vs 2.5 m, p=0.001). No other significant differences were found. 55% pt that progressed received second line. After second PD, 50% had subsequent treatment. 36% pt needed dose-reduction and 73% pt had treatment discontinuation, mostly due to PD (67%). 27% presented grade 3-4 toxicities, mostly fatigue (11%) and hematological (6.6%). There were no toxic deaths.

In our small, retrospective series, we showed that elderly pt benefit from systemic treatments in first-line with an acceptable toxicity profile. Basal LDH and PS were related with better outcomes.

Targeting mitochondrial metabolism to explore intratumor heterogeneity and therapy resistance in melanoma

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The use of BRAF inhibitor (BRAFi) has revolutionized melanoma therapy, although relapse is almost certain. Besides controlling cell proliferation and survival, BRAF oncogene is linked to metabolic reprogramming. Thus, cancer metabolism can be considered as an emerging source of new targets for therapy. Melanoma adopts a Warburg phenotype which makes the cells dependent on aerobic glycolysis for survival and proliferation. BRAF controls the expression of MITF that drives overexpression of PGC1-alpha in a subset of melanomas. PGC1-alpha promotes mitochondrial biogenesis and oxidative phosphorylation (OXPHOS), placing mitochondria as a central organelle. Intratumor microenvironment propitiates phenotypically distinct cell subpopulations that contribute to therapeutic resistance. However, as mitochondrial metabolism can control invasiveness or resistance in these subpopulations is poorly understood and, exploring the molecular events that drive melanoma plasticity is critical to improve the treatment approach. Here, we stochastically isolated two clonal subpopulations from human metastatic melanoma cell line WM164 (CI and CII), mimicking the initial clonal subpopulations in a tumor. Our results showed that CI is more proliferative and appear to be less sensitive to BRAFi, while CII is the opposite. Also, we demonstrated that CI uses preferably OXPHOS than glycolysis and showed identical profile for oxygen consumption rate when compared to WM164 vemurafenib resistant cell line. Thus, oxidative metabolism can be considered as an adaptive mechanism that limits the efficacy of BRAFi. Of relevance, CI presents higher expression of MITF, SOD1 and SOD2 than CII. Ultimately, the heterogeneity within a tumor uses mitochondrial metabolism to evade BRAFi therapy.

Melanoma-secreted factor MIDKINE drives immune checkpoint blockade resistance and predicts clinical outcome

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Cutaneous melanoma is the most lethal form of skin cancer, characterized by its metastatic potential and a remarkable ability to evade the immune system. Therapies aimed at the deactivation of intrinsic mechanisms of immunosuppression have improved clinical

response rates, but about 40-50% of patients still succumb to metastatic disease. Primary resistance to immunotherapy is often observed in patients with low immunogenic tumors (cold tumors), or with lesions infiltrated with immune suppressive cells, such as tumor-associated macrophages (TAMs), T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Yet, mechanisms that define tumor immunogenicity, and more importantly, biomarkers to predict clinical responses in patients, are still pending needs in the field. We have previously identified a melanoma-secreted protein, called MIDKINE (MDK), with critical roles in lymphangiogenesis and metastasis. We have now identified a new MDK-related transcriptomic gene signature with a high significant correlation to survival in melanoma and other tumor types. This MDK-associated signature was found correlated to increased immune cell infiltration, in particular, of MDSCs and Tregs. Mechanistically, we assigned this immunomodulatory function of MDK to a secretory program acting both on tumor cells (via ALK) and on myeloid cells (via STAT3). Gain-of-function assays demonstrated that MDK blunts the response to immune checkpoint blockers actively pursued in the clinic. The physiological impact of these results was further strengthened by finding that MDK expression predicts resistance to anti-PD1-based treatment in two independent cohorts of melanoma patients. These results provide insight on long-pursued mechanisms of tumor-immune evasion in melanoma, and uncovered MDK as a tractable biomarker for the response to clinically relevant immunomodulatory agents.

Chemically enforced differentiation leads to melanoma tumor control by overcoming malignant cell state transitions and restoring tumor immunogenicity

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Phenotypic heterogeneity and transitions between cell differentiation states represent major drivers of therapy resistance in melanoma. Expression of the H3K4 demethylase and pRB-binding protein JARID1B/*KDM5B* normally follows a dynamic equilibrium across melanoma cells, but its interconnection with cellular differentiation remained elusive so far. The highly JARID1B-expressing cell state is slow-cycling and becomes enriched after short-term drug treatment, whereas under persistent drug-exposure, melanomas decrease JARID1B expression again to re-enter cell cycling for long-term tumor repopulation. Here, we could overcome melanoma cell state transitions by biologically and chemically enforced homogenization of the JARID1B^{high} phenotype, which induced tumor growth arrest also in MAPKi-resistant cells. Unexpectedly, JARID1B-homogenized melanoma cell populations left their mesenchymal gene programs towards a differentiated, MITF^{high}/AXL^{low} profile including reconstituted immunogenicity. Mechanistically, JARID1B represents an integral checkpoint for coordinating both the immune-differentiation phenotype of melanoma cells via transcriptional reprograming and the cell cycle via stabilization of hypophosphorylated pRB plus attenuation of cytokinetic abscission. Thus, the JARID1B-dependent ability of melanoma cells to dynamically switch between differentiation, cell cycle, and immune states can be chemically targeted to control tumor growth also in the MAPKi-resistant scenario and to prime melanoma for immune therapies.

Sentinel Lymph Node Biopsy in 570 Chinese Acral and Cutaneous Melanoma Patients
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Background: The current study was performed to identify prognostic factors for prediction of sentinel lymph node (SLN) metatasis in Acral and Cutaneous Melanoma Patients.
Methods: The records of 570 melanoma patients undergoing SLN biopsy in Fudan University Shanghai Cancer Center (FUSCC) from September 2009 to August 2017 were reviewed. Clinicopathologic data including age, tumor maximum diameter, Clark level, Breslow level and ulceration were analysed for the prediction of SLN metastasis. Univariate chi-square and multivariate logistic regression analyses were performed to identify predictors of positive SLNs.
Results: Positive SLN(s) were found in 150 (26.32%) patients. Univariate analysis showed that Clark level (p=0.033) and ulceration (p=0.017) were associated with SLN metastasis. By multivariate analysis, only Clark level (p=0.038) was independent predictive factor for SLN metastasis. Among all the 570 patients, 378 were acral melanoma and 192 were cutaneous melanoma. In acral melanoma,

Clark level was also the only independent predictive factor for SLN metastasis (p=0.002). However, in cutaneous melanoma, ulceration was the only independent predictive factor for SLN metastasis (p=0.038).

Conclusions: While Clark level was dominant predictor for SLN metastasis in acral melanoma, ulceration may be more important in predicting SLN metastasis in cutaneous subtype.

Margin width in plantar melanoma: Is a 2cm resection margin mandatory?

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Background: There is no consensus for resection margin in plantar melanoma. The current study was to investigate the impact of different resection margins and other prognosticators on prognosis of plantar melanoma.

Methods: The records of all plantar melanoma patients undergoing surgery in Fudan University Shanghai Cancer Center from September 2009 to August 2017 were reviewed. All patients were followed-up to March 1, 2018 or until death. Log-rank analysis was used to evaluate the prognostic value of margin width (<2cm vs ≥2cm) and other factors including age, Clark level, Breslow thichness, N stage and ulceration. Resection margin and other prognostic factors obtained from the univariate Log-rank analysis were included for multivariate cox regression analysis.

Results: A total of 328 patients enrolled. The median age at diagnosis was 51 years (25%-75%, 51-70 years). The median follow-up time was 28 months (25%-75%, 14-43 months). At the end of follow-up, 102 patients' events were recorded including 60 deaths. In the univariate analysis, Clark level (p=0.030), Breslow thichness (p<0.001) and N stage (p<0.001) were prognostic factors for patients' disease free survival (DFS); Breslow thichness (p<0.001) and N stage (p<0.001) were prognostic factors for patients' overall survival (OS). Margin width had no effect on patients' DFS (p=0.926) or OS (p=0.533). In the multivariate analysis, Breslow thichness (p=0.042 for DFS; p=0.020 for OS) and N stage (p<0.001 for DFS and OS) were both independent prognostic factors for patients' DFS and OS. Margin width still had no effect on patients' prognosis (p=0.879 for DFS; p=0.494 for OS).

Conclusions: Breslow thickness and N stage were independent prognostic factors for plantar melanoma patients' DFS and OS. Margin width of <2cm or ≥2cm had no effect on prognosis of plantar melanoma, which warrants further study to obtain best oncologic and functional outcome.

Phenotype switching as a predictor marker for sensitivity to BRAF and MEK inhibitors

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Targeted therapy in melanoma has been a great success in extending progression free survival and overall survival for melanoma patients. These include BRAF inhibitors for patients with a BRAF V600 mutation and MEK inhibitors for patients with an NRAS G12 or Q61 mutation. However, only about 50 % of patients respond to single BRAF inhibitor therapy and about 70% respond to combination BRAF and MEK inhibitor therapy. To elucidate possible biomarkers for response, we performed viability assays, RNAseq, and targeted DNA sequencing on a panel of 70 early passage melanoma cell cultures from the URPP biobank of the University Hospital Zurich. Early passage melanoma cell cultures came from patients naïve, on, and progressing from targeted therapy. *In vitro* viability assays with BRAF or MEK inhibitor revealed some cell cultures were resistant even though the patient was naïve to targeted therapy, in addition some cell cultures established from progressive patients were not resistant to BRAF or MEK inhibitor *in vitro*. Supervised hierarchical analysis of the RNAseq data based on *in vitro* resistance to BRAF or MEK inhibitor revealed an enrichment for the phenotype switching signature. Supervised analysis of the targeted DNA sequencing data revealed no hotspot mutation or gene that could be predictive of sensitivity to BRAF or MEK inhibitors. We conclude that the phenotype switching signature could be a useful predictive tool to stratify patients for targeted therapy.

Patterns of response with talimogene laherparepvec (T-VEC) in combination (combo) with ipilimumab (ipi) or ipi alone in patients (pts) with metastatic, unresectable melanoma (MEL)

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Background

In a phase 2 trial involving pts with metastatic, unresectable MEL, the combo of T-VEC and ipi resulted in a significantly higher objective response rate (ORR) vs. ipi alone (odds ratio, 2.9; 95% Cl, 1.5–5.5; *P* = .002) (Chesney et al. *J Clin Oncol*. 2017). Here, we describe the patterns of response.

Method
198 pts with unresectable stage IIIB-IV MEL were randomized 1:1 to T-VEC plus ipi (n = 98) or ipi alone (n = 100). Pts who had an OR were evaluated for progression prior to response (PPR), defined as ≥ 25% increase in tumor burden from baseline. Tumor response was assessed every 12 weeks (wks) after treatment (tx) started. Pts with PPR were further classified according to whether PPR was due to increase in existing lesions or contribution of addition of new measurable lesions; pts without PPR were further classified according to whether they responded within or after 6 months.

Results
38/98 (39%) pts in the combo arm and 18/100 (18%) pts in the ipi arm had an OR. 7/38 (18.4%) responders in the combo arm had PPR (5 due to increase in existing lesions and 2 due to new measurable lesions). 1/18 (5.6%) responders in the ipi arm had PPR in existing lesions. Most PPR was shown by the 1st tumor assessment at wk 12. Majority of patients without PPR (30/31 in combo arm; 16/17 in ipi arm) responded within 6 months.

Conclusions
ORR was higher in the combo arm despite higher incidence (inc) of PPR. The PPR inc with the combo is lower than that of 48% reported for T-VEC monotherapy but higher than that reported for checkpoint inhibitors (CPIs) (Andtbacka et al. *Ann Surg Oncol*. 2016;23:4169–77; Hodi et al. *J Clin Oncol*. 2016;34:1510–7). These data reinforce the use of immune-related response criteria and support the T-VEC tx through initial progression in MEL when given in combo with a CPI.

Functional testing of MTAP haplotypes demonstrates differential expression, signalling and pigmentation effects in primary melanocytic cells

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MTAP, a housekeeping gene converts methylthioadenosine (MTA), a by-product of polyamine synthesis into adenine and methionine. MTA levels affect cellular signaling and function not just in cancer but also surrounding lymphocytes and stromal fibroblasts, characteristic of malignant melanomas. The MTAP locus is strongly associated with nevus count and melanoma in European derived populations. A *MTAP* tagging haplotype consisting of four SNPs, rs4636294*A/G located in the 5' UTR, rs935055*G/C and rs7023329*A/G in intronic region and rs7023954*G/A a coding change V56I, was associated with increased adult nevus counts and melanoma risk. The A-G-A-G (H) risk and G-C-G-A (WT) protective haplotypes respectively are at approximately equal frequencies in European populations. The functional elements associated within these haplotypes were examined, notably the rs935055 SNP resides in a DNAase hypersensitive site associated with H3K27Ac modified chromatin and transcription factor (TF) binding sites, making it a lead candidate as a causative SNP. Luciferase reporter assay based testing of these SNPs indicate that rs935055*G (H) has 3fold activity over the WT reporter. Using human foreskin-derived melanocytic strains as a primary model for investigation, we have explored the correlation between MTAP haplotypes and candidate TFs (MITF, and BRN2) and signaling cascades. These have been further tested for pigmentation, proliferation and migration attributes owing to altered MITF-BRN2 levels. This study indicates the effect of differential protein methylation owing to genotypic variation in MTA levels. The effects of endogeneous MTA on PRMTs as presented in this study strongly support migratory phenotype as well altered p53 and interferonγ signaling of melanocytic cells thereby promoting melanoma incidences based on their MTAP haplotype.

Convergence of Keratinocyte Stem Cell Governance, SHH and Ribosomal Biogenesis Pathways in Human Nevogenesis
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Melanocytes can group together and form nevi, commonly thought to form due to intrinsic somatic mutations involving MAPK pathway activation. Perturbation of Keratinocyte stem cells could affect the biology of melanocyte stem cells which also reside in hair follicles. However, the role of the microenvironment, especially keratinocytes in nevogenesis is elusive and rarely studied. Melanocytes proliferate during the hair follicle growth phase and in basal cell carcinoma, allowing us to construct keratinocyte gene clusters correlated with melanocyte activation. We first considered genes which when mutated in keratinocytes in mice lead to nevogenesis. Using the human Genotype-Tissue Expression (GTEx) skin database we analyzed gene expression data across many individuals and found that the expression of these nevus-causing genes in keratinocytes is correlated with that of specific keratinocyte cytokines (KITLG, HGF, FGF2 and EDN1), and melanocyte activation. These cytokines have pleiotropic effects on melanocyte-specific and pigmentation genes, and also influence mast cell gene expression. Here, we reveal five classes of keratinocyte genes which via germline genetic variation in humans influence melanocyte activity. These include genes involved in SHH signalling, structural keratins, ribosomal biogenesis, and stem cell governance. In agreement with the finding of *KITLG* linked to nevogenesis in human genome wide association studies, our results provide evidence that specific keratinocyte genes and cytokines are part of networks that in human skin may drive or exacerbate nevus development.

Compositional differences in the gut microbiome are associated with distinct tumor and systemic immune profiles in melanoma

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Emerging evidence suggests a strong link between gut microbiota and response to immune checkpoint blockade. Preliminary evidence suggests that a more "favorable" gut microbiome is associated with a higher density of CD8+ T cells in the tumor, and more effector T cells in the periphery, however additional insights are needed to understand its full impact.

To address this, we performed studies in patients and pre-clinical models using flow cytometry and cytometry by time-of-flight (CyTOF). Flow cytometry in germ-free (GF) mice transplanted with responder (R) (n=5) vs nonresponder (NR) (n=6) fecal microbiota transplant (FMT) revealed differential immune signatures between R-FMT and NR-FMT, with a higher density of innate effectors and increased PD-L1 expression in mice receiving R-FMT. In contrast mice receiving NR-FMT had an increased density of regulatory T cells and TH17 T cells (p<0.01).

We next used CyTOF to more deeply phenotype the immune cells. We initially performed this in samples from R and NR patients on anti-PD-1 with available gut microbiome data (n=6, 3 in each group) Preliminary data from a subset (n=1/group) showed that that R to anti-PD-1 (with more favorable microbiome) had an increseaed frequency of tumor infiltrating B-cell and CD4+ T-cell populations versus NR. NR (with a less favorable microbiome) had a higher frequency of myeloid and T reg populations.

Following this, studies were performed in GF mice receiving R-FMT with a subset treated with probiotics to disrupt the microbiome (n=12). Tumors were profiled by CyTOF and 17 distinct CD45+ clusters/ populations were identified in the TME, with several populations significantly modulated with probiotic use (including two myeloid specific clusters). Additional studies are underway, but these studies provide additional evidence on how gut microbiota may influence response.

DANTE: A new randomised trial to evaluate the treatment <u>D</u>uration of <u>AN</u>ti-PD1 monoclonal antibody <u>T</u>reatment in patients with metastatic m<u>E</u>lanoma

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Patients with metastatic melanoma are treated with anti-PD1 antibodies. Unlike the anti-CTL4 antibody, ipilimumab, which is given for a fixed duration of 12 weeks, anti-PD1 antibodies are licensed for use for as long as there is clinical benefit, or until treatment is not tolerated. Many responses occur in the first year and can continue even after treatment is stopped for toxicity or patient choice. We hypothesize that continuing treatment beyond 1 year is unnecessary, exposing patients to the risk of developing immune-related toxicities and incurring considerable costs for the NHS.

The UK NCRI Skin Cancer Clinical Studies Group developed the DANTE trial to evaluate the duration of anti-PD1 monotherapy. It opened in summer 2018 and will run in >40 UK sites.

Patients receiving anti-PD1 monotherapy are registered into DANTE within 1 year of starting treatment. 1208 patients who remain progression-free at 1 year are then randomised to either a) stop treatment (with the option to restart anti-PD1 therapy or commence other treatment on progression) or b) continue until disease progression/unacceptable toxicity or a minimum of 2 years in the absence of progression/toxicity. Patients are followed up for 4 years. The primary outcome is progression-free survival. Secondary outcomes are quality of life, overall survival, response rate and duration, safety, cost effectiveness and patient acceptance of randomisation. Planned translational studies will study response and toxicity biomarkers.

The outcomes of DANTE will be of national and international importance in melanoma and other cancers.

Comprehensive genomic profiling of acral and mucosal melanomas identifies potential therapeutic strategies

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Non-sun exposed melanomas, such as acral and mucosal (A/M) subtypes, account for ~ 10% of all melanomas. Due to their rarity, the genomic landscape of A/M melanoma is incomplete and targeted therapies for A/M melanoma are not well explored. We performed comprehensive genomic profiling (CGP) on 155 melanomas, including 54 A/M melanomas. Cancer associated genes (n=395) were interrogated for genomic alterations (GAs) (base substitution, insertions/deletions, copy number alterations, and gene rearrangements) by next generation sequencing. We identified 102 genes with GAs present in at least 5% of A/M melanomas. Forty genes were recurrently amplified including *MYC*, *CCND1*, *CDK4*, *MDM2*, and *CRKL*. Three genes (*AKT1*, *KDR*, and *CDKN2A*) had recurrent rearrangements. Frameshifts, missense mutations, and stop gains in DNA damage repair genes were common. Gene ontology analysis identified 13 pathways enriched in A/M melanomas, including MAPK, cell cycle, PI3K/mTOR, and p53/DNA damage. We generated four A/M cell lines from patient tumors and tested treatment response to 10 different inhibitors targeting each of these pathways. A/M melanoma cell lines showed sensitivity to buparlisib (PI3K), JQ1 (*MYC* transcription), dasatinib (*CRKL* amplification), and cell cycle inhibitors, but were less sensitive to everolimus (mTOR) and DNA damage modulating inhibitors. Additionally, we found A/M cell lines were highly sensitive to MAPK inhibition, and an acral melanoma cell line showed synergistic effects between trametinib (MEK inhibitor) and LY3009120 (pan-Raf inhibitor). One acral melanoma patient with *NRAS* mutation was treated with a pan-Raf inhibitor and experienced a complete and ongoing response lasting over five years. In summary, we have expanded the molecular landscape of A/M melanoma and identified several potential therapeutic strategies for treating A/M melanoma.

The tumor suppressor role of AMBRA1 in melanoma

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Autophagy is a degradation process activated by the cell to recycle cellular components and eliminate damaged organelles in order to guarantee its survival in adverse conditions. Dysregulation of autophagy contributes to the development and progression of melanoma and plays a crucial role in melanoma response to treatment. The aim of this study is to decipher the role of the autophagy protein AMBRA1 (activating molecule in Beclin1-regulated autophagy) in melanoma development. At variance with other autophagy genes, we discovered that AMBRA1 controls cell proliferation through the regulation of c-Myc activity, causing the spontaneous tumor insurgence in Ambra1 haplo-insufficient mice, as well as the increasing size of Ambra1-deficient tumor xenografts (Cianfanelli et al., 2015). These observations point out AMBRA1 as a new oncosuppressor. In line with these findings, we used cell/animal system-based studies to show that loss of Ambra1 impacts on genomic stability. In addition, using a large panel of human melanoma cells as well as the genetically engineered mouse carrying Braf^{V600E} mutation in combination with Pten deletion - which recapitulates hallmark features of the human disease - we are studying the involvement of Ambra1 in melanoma. Our preliminary data indicate a potential role of Ambra1 in melanoma ontogenesis and progression. Further studies will help determining if Ambra1 can be exploited as new target in melanoma therapy.

Cianfanelli, V., et al. (2015). AMBRA1 links autophagy to cell proliferation and tumorigenesis by promoting c-Myc dephosphorylation and degradation. Nature Cell Biology, 17(1), 20–30. https://doi.org/10.1038/ncb3072

Multi-level analysis of AMBRA1 and characterization of its mutations in melanoma.

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Intrinsically disordered proteins (IDPs) are components of the cellular signaling machinery which actively participate to the assembly of complexes and to the control of several cellular processes. To add complexity to this regulatory network, IDPs can combinedly undergo post-translational modifications (PTMs) which can further regulate and affect protein-protein interactions. Among these proteins is AMBRA1 (activating molecule in Beclin1-regulated autophagy), whose multifaceted role in cells consists in the regulation of many key processes tightly (de)regulated in cancer, including cell proliferation and autophagy, the recycling process that, degrading cellular components through the lysosome factory, is involved in both carcinogenesis and tumor progression/metastasis. Though

molecular aspects of melanoma are still in need of further elucidations, different risk factors have been associated with its occurrence, mainly relying on genetic alterations. Among these, somatic mutations in BRAF or NRAS, have been identified and deeply investigated so far. Here, we applied an integrative bioinformatic approach by means of two cancer genomics platforms (cBioPortal and COSMIC) to identify mutations of AMBRA1 in melanoma and to assign them a predicted pathogenicity score (REVEL score). Interestingly, we observed that AMBRA1 is highly mutated in melanoma, with mutations mainly mapping in proximity or within regions involved in protein interaction as well as at PTM sites. On the basis of the REVEL score, a subset of such mutations (hence predicted to have either a functional or structural impact on the protein) were selected and specific tools developed in order to study the *in vitro* relevance on the pathways orchestrated by AMBRA1 in melanoma.

Regions of Copy Number Variation (CNV) Identified in Primary Melanoma

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CNV is defined as a DNA segment of 1kb or larger whose CN differs from a reference genome. Fehrmann et al. (2015) analysed 77,840 tumour expression profiles and showed that CN identified recurrently disrupted genes in genomically unstable cancers. Here, we report the first large scale CN study of primary melanoma. NGS data from 303 formalin fixed paraffin embedded samples from the Leeds Melanoma Cohort (LMC) were generated. Libraries were generated by random shearing and then sequenced (1.7x). Problematic regions in the genome were identified and excluded in the analysis to reduce chance of false positives; CN was generated by read count adjustment jointly of sequence mappability and GC-content and comparison Caucasian genomes from the 10k Genome Project. A total of 13 % of the autosomal genome was excluded on the basis of incomplete or complex structure. *GISTIC2.0* (Mermel et al., 2011) identified significantly deleted or amplified regions in the genome. Overall, we found a highly similar pattern of CN changes between the LMC primaries and TCGA metastases. For regions with deletions, 9p21.3 was identified as the most significantly deleted in both datasets (q-value=1.2×10^{-115} and 8.2×10^{-218} for LMC and TCGA respectively). For amplifications, LMC analysis identified 7p22.1 (q-value=1.7×10^{-14}) as the region that is most significantly amplified while TCGA identified 11q13.3 (q-value=2.0×10^{-15}). Overall, a lower proportion of samples with deletion or amplification in LMC was noted as compared to TCGA. We are investigating whether the observed differences are due to the type of platforms used in generating the data (NGS vs SNP array), data pre-processing, analytical processes, and/or differences on stage of melanoma (LMC has only primaries while TCGA CNV data has only metastatic melanoma samples).

Myeloid-Derived Wnt5a Regulates the Phenotype of Melanoma

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The change from a proliferative, therapy sensitive state to an aggressive, therapy resistant phenotype is characterized through a change in WNT ligand signaling from the canonical β-catenin dependent pathway to the non-canonical β-catenin independent pathway respectively. Wnt5a is a glycoprotein that signals through the non-canonical pathway and induces a highly invasive cell state, is highly-expressed in metastatic melanoma and correlates with Vemurafenib and immune-mediated resistance. Our data, for first time, now suggest that myeloid derived suppressor cells (MDSCs), a key subset of potent immunosuppressive cells frequently associated with aggressive cancers, produce a major proportion of the Wnt5a in the tumor microenvironment increasing cancer invasiveness. This finding not only links two potent drivers of tumor progression but augments the role that MDSCs play in melanoma metastasis.Using novel transgenic animals with myeloid-specific Wnt5a deletions, we demonstrate a clear decrease in the Wnt5a expression within the TME. We then demonstrated that WNT5A-deficient MDSCs caused a significant decrease in circulating T-cells, tumor-infiltrating MDSCs and Tregs. Supporting our hypothesis that Wnt5a promotes a switch from proliferation to an invasive melanoma cell state, myeloid-specific Wnt5a deletion caused a significantly increased tumor growth in vivo, with the lack of MDSC infiltration demonstrating a strong negative correlation to tumor volume. Tumor-infiltrating MDSCs from control animals demonstrated a strong positive correlation with Tregs and this correlation was completely ablated in animals with Wnt5a-negative MDSCs. Overall, our data suggests that while MDSCs provide an immunosuppressive environment, they may have an additional function as the major source of Wnt5a in the tumor microenvironment. These two functions likely synergize to drive a highly metastatic and therapy-resistant phenotype.

Metabolic targeting as a strategy to overcome targeted therapy resistance

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Cancer cell heterogeneity poses a major challenge in the treatment success of metastatic melanoma. Targetable driver mutations are well characterized, but phenotype switching provides plasticity to melanoma cells to escape treatment. While melanoma cells in a melanocytic expression state are sensitive to targeted therapies, mesenchymal signature cells (e.g. high TGFbeta signalling, WNT5a

and AXL) are treatment resistant. We have analysed the metabolome & transcriptomes of these two distinct phenotypes, and consistent with the Warburg hypothesis in which tumors produce a large proportion of energy through glycolysis, melanocytic melanoma cells have elevated glycolytic metabolites. In contrast, mesenchymal melanoma cells have lower levels of glycolytic metabolites, are at their maximal mitochondrial capacity, and have high levels of reactive oxygen species (ROS). In a high-throughput screen of 960 compounds, one lead compound in particular effectively and selectively targeted the viability of mesenchymal melanoma cells. Further targeted proteomics and in vitro analysis revealed the compound to be a ROS inducing agent that targets the mitochondrial respiratory chain. Treatment-resistant, NRAS-mutated melanoma cells showed a high degree of vulnerability to ROS induction. The sensitivity to the ROS inducer was confirmed in 3D spheroids, patient-derived ex vivo tumor models, and *in vivo* animal models. We found that by inducing a phenotype switch in melanocytic melanoma cells, we could alter the metabolic cell phenotype and sensitize the cells to our compound through ROS induction. These findings link the transcriptional cell state to a metabolic phenotype that can be inhibited by our small molecule. This suggests a novel strategy of "metabolic targeting" to prolong the efficacy of MAPKi-inhibitors and delay resistance onset in metastatic melanoma therapy.

Thrombospondin-1 fragments in platelet-poor plasma – a new biomarker for metastatic uveal melanoma

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Uveal melanoma is the most common intraocular malignancy in adults. Approximately 50% of all patients with uveal melanoma die from liver metastases that frequently appear more than 5 years after discovery of the primary tumor. Currently, lactate dehydrogenase (LDH) is the only validated biomarker for uveal melanoma, but it has poor sensitivity and specificity. To identify metastases as early as possible and to monitor treatment, a better biomarker is needed.

Here, we evaluated levels of immunoreactive thrombospondin-1 fragments in platelet-poor plasma as a biomarker for uveal melanoma and compared it to LDH. 10 patients with metastatic uveal melanoma were included in the study. A control group of 14 healthy individuals was used. Blood samples were drawn from each individual and platelet-poor plasma was promptly prepared. Platelet-poor plasma is required because thrombocytes contain high levels of thrombospondin-1. The concentration of thrombospondin-1 fragments was measured using ELISA.

Thrombospondin-1 fragment levels had a sensitivity of 60% to identify patients with metastatic disease, which was identical to LDH. Furthermore, the concentration of thrombospondin-1 fragments was higher in patients with extensive disease compared to those with limited disease. The difference was borderline significant ($p = 0.067$) in our small group of patients. In controls, all subjects with increased thrombospondin-1 fragments were women below 50 years of age. Importantly, combined analysis of both LDH and thrombospondin achieved a high sensitivity of 80%.

Thrombospondin-1 fragment levels in platelet-poor plasma, especially in combination with LDH, are a promising biomarker for metastatic uveal melanoma. Some healthy women under 50 years of age have naturally high thrombospondin-1 fragment levels, possibly owing to hormonal or other variations during the menstrual cycle.

Concordance of BRAF mutations in circulating tumor DNA identified using biochip assay compared to tissue

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Curculating tumor DNA (ctDNA) is released to blood from tumor cells undergoing apoptosis, necrosis and active secretion. Detecting mutations in ctDNA can be used as an alternative to tissue testing. The analysis of ctDNA presents great diagnostic and prognostic potential in the process of cancer treatment.
For analysis biomarkers in ctDNA we used a biochip for detection mutations in BRAF gene (V600E, V600M, V600K, V600R, V600D) which we developed earlier. For preferable amplification of mutated over WT DNA we used nested LNA clamp PCR. PCR fragments were labeled via incorporation of Cy5-dTTP and hybridized with specific oligonucleotides immobilized on a biochip. Method allows detecting 0.05% mutated DNA in a background of WT DNA.
We tested 42 plasma samples from melanoma patients with known BRAF status: 14 tumors were WT and 28 ones were mutated. The ctDNA was isolated from 2-5 ml of plasma collected prior to surgical intervention. In 19/28 BRAF-positive patients the consistency of results between ctDNA and tumor DNA was observed. Most of patients (8/9) with discordant genotyping results had no metastases in contrast to patients with coincidental results. We detect BRAF mutations in two ctDNA samples from 14 BRAF WT patients. We checked these result with droplet digital PCR: in one ctDNA sample mutation was confirmed, in the second sample the ddPCR detected positive drops, but their number isn't enough to conclusion that sample is mutated. In these two cases, the discordance between ctDNA and the tumor is most likely due to the heterogeneity of the tumor.

The biochip assay is a sensitive method for mutation detection in ctDNA. Analysis of ctDNA can be used in conjunction with traditional tumor analysis to prevent a false negative result as a result of tumor heterogeneity. This work was supported by Grant of the President of the Russian Federation (# MK-2519.2017.4).

Microarray gene expression of the primary tumor predicts sentinel lymph node disease

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Background: Sentinel lymph node dissection is used in melanoma to stage the lymph nodes and direct therapy. Despite the accepted pathologic features that lead to nodal sampling, 80% of patients subjected to the invasive staging procedure are found to have a negative sentinel lymph node (SLN). We hypothesized that the primary tumor microenvironment influences metastatic potential and examined 79 immunomodulatory genes relevant to cancer as a method to predict lymph node metastases.
Methods: 37 patients with primary melanoma (thickness 0.15-5mm) and no gross nodal disease underwent surgical excision of the primary and SLN biopsy with median follow-up 38 months. Based on microarray analysis of the primary tumor, differential gene expression of 79 immunomodulatory genes was compared between patients who had positive SLN (n=5) and those who did not (n=32).
Results: The patients with positive SLN had primary tumors with significantly increased ulceration rates (p=0.02) and higher mitotic index (p=0.01) compared to those with negative SLN. There were no differences in age, gender, Breslow thickness, regression, or site of primary. Of the 79 genes analyzed, ACTA2, which encodes actin, was significantly underexpressed in the positive SLN group with a differential expression fold change (DEFC) of 0.71 (p<0.01). As a predictor of SLN positivity, ACTA2 had a strong area under the curve (AUC) of 0.85. Patients with low ACTA2 expression in the primary had 5-year survival of 63% compared to 97% for those with high ACTA2 expression (p=0.58).
Conclusion: Diminished ACTA2 expression in primary melanoma specimens is associated with positive SLN. Conversely, increased ACTA2 expression is associated with negative SLN and better patient outcome. Examination of ACTA2 expression in the primary melanoma biopsy could be used for risk stratification and identify low-risk patients who might not need SLN sampling.

Dissecting the role of IFNγ in adaptation and therapy resistance in melanoma

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IFNγ is a key cytokine, regulating a plethora of biological processes, including antitumor immunity. Its role in tumor immunology is however contradictory, exerting anti-tumor effects early in tumor development but ultimately promoting tumor immune evasion. Its involvement in immunotherapy resistance is also extremely complex and is potentially mediated through multiple cellular processes. Stat1, an essential intracellular transducer of IFNγ, has also been linked to resistance to conventional genotoxic therapy. In this study, we aimed to generate further insights into the role of sustained IFNγ signaling in adaptation and acquired resistance to therapy in melanoma. To this end, melanoma cell lines were treated with increasing doses of IFNγ and the levels of IFNγ pathway activation were assessed, showing that established melanoma cell lines retain responsiveness to IFNγ. The effects of chronic IFNγ treatment in gene expression of melanoma cell lines were assessed by RNA sequencing. Interestingly, pathway analysis of differentially expressed genes revealed oxidative stress response as well as DNA damage and repair pathways among the top upregulated pathways in IFNγ treated cells. In line with this, gene set enrichment analysis showed enrichment of DNA damage response and repair related signatures in genes upregulated by IFNγ treatment. The clinical relevance of these findings is highlighted by the fact that high expression of DNA damage response signature correlates with poor prognosis and relapse in melanoma patients. Next, we showed that chronic IFNγ exposure induces DNA damage in melanoma cell lines, patient-derived short-term cultures and zebrafish melanomas. Finally, we showed that chronic IFNγ exposure renders melanoma cells resistant to DNA-damage induced cell death and we identified *IGTG1* as an important mediator of the IFNγ-driven resistance to DNA damaging effects of genotoxic drugs.

Melanoma dormancy and the aged tumor microenvironment: WNT5A drives disseminated melanoma cell dormancy

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Metastasis is the most common cause of melanoma-related deaths, with treatments failing to provide a durable response. Tumor dormancy describes a slow-cycling population of cells seeded within visceral organs that are often undetectable and resistant to therapy. Given the lack of knowledge of dormant pathways within melanoma or how cells emerge from dormancy, there are few clinical markers for the identification of dormant micrometastases. Aging is a key prognostic factor in cancer that we hypothesize can reprogram the stromal microenvironment to allow a more growth-permissive tissue for melanoma metastases in distant organs. Our studies find that while melanoma cells implanted into skin of young and aged mice disseminate into tissue at a similar rate, these cells form significantly more overt metastases in the aged lung. WNT5A is a driver of melanoma dissemination and is key in inducing a slow-cycling resistant phenotype, thus we hypothesized it may also promote dormancy following dissemination. Our studies show that

WNT5A increases the expression of key dormancy pathways (AXL, NR2F1 and p38) and that young lung fibroblasts promote these pathways and inhibit melanoma growth. Proteomic analysis of young/aged lung fibroblasts reveals that indeed, aged fibroblasts secrete increased levels of the WNT5A antagonist sFRP1 and the tumorigenic factor SERPINE1. Treatment of melanoma cells with sFRP1 and SERPINE1 synergizes to increase melanoma growth by downregulating WNT5A and dormancy associated gene expression. Overall, these studies find that contrary to the changes observed in the skin microenvironment, the young lung microenvironment promotes melanoma dormancy via upregulation of WNT5A and dormancy markers, while the aged lung microenvironment induces an emergence from dormancy by increasing secreted factors that inhibit WNT5A and dormancy associated pathways.

Tumoural melanosis: in what clinical contexts does it occur and what does it mean for the patient?

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Tumoural melanosis is a histological term to describe a nodular aggregation of macrophages containing melanin pigment (melanophages) that is devoid of melanocytes. It most often arises in the dermis, where it may be appreciated clinically as a pigmented lesion, but it can also be found at other sites, such as lymph nodes. Although it has been rarely reported in association with other cutaneous tumours such as pigmented basal or squamous cell carcinomas, most cases are thought to represent regressed melanoma. Despite its increasing frequency in the current era of effective systemic therapies for melanoma, tumoural melanosis has not been previously analysed in a large series.

We undertook a retrospective review of all histopathological diagnoses of tumoural melanosis reported at the Royal Prince Alfred Hospital from 2006-2018. Tumoural melanosis occurred in patients with a median age of 65 years (range 22-85) with no sex preponderance. The most common organ of involvement was the skin (78%), with a third of all cases based in the lower limbs. Most patients previously (89%) or subsequently developed histologically confirmed melanoma. Ultimately, 78% of patients developed melanoma in the same region as the tumoural melanosis and 65% showed evidence of metastatic melanoma.

Patients with tumoural melanosis were distributed across three different clinical groups: no history of melanoma (n=6), a previous or concurrent diagnosis of melanoma (n=28), and patients who had received local or systemic therapy for melanoma (n=49). Whilst tumoural melanosis can signify a response to therapy, particularly in the neoadjuvant setting, in untreated patients it is associated with a high rate of metastatic melanoma and death. Recognising tumoural melanosis and distinguishing the clinical scenarios in which it may arise can provide useful information for patient management.

Oxidative Phosphorylation (OXPHOS): A Feature, Driver, and Therapeutic Target in Melanoma Brain Metastases (MBMs)

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There is a critical unmet need to improve the understanding and treatment of MBMs. While recent studies have examined signaling pathways and immune cells/regulators in MBMs, very little is known about the metabolic features and dependencies of these tumors. Thus, we performed RNA-sequencing (RNA-seq) analysis on (i) 88 surgically resected formalin-fixed, paraffin-embedded MBMs, (ii) 50 patient-matched extracranial metastases (ECMs), and (iii) 54 unmatched primary tumors (PTs). Pathway analysis identified OXPHOS as the metabolic pathway most significantly enriched in MBMs compared to both patient-matched ECMs and PTs. RNA-seq analysis of MBMs, ECMs, and PTs from a previously described RCAS-TVA autochthonous murine model of spontaneous melanoma brain metastasis similarly showed upregulation of OXPHOS in MBMs. Further, increased expression of OXPHOS genes was detected in intracranial (IC) versus subcutaneous (SQ) xenografts of 5 different human melanoma cell lines. Increased OXPHOS in the IC xenografts was confirmed by direct metabolite analysis and [U-^{13}C]-glucose tracing analysis. The functional significance of OXPHOS was tested using IACS-010759, a potent OXPHOS inhibitor currently in phase I clinical trials. Treatment of the autochthonous MBM model with IACS-010759 significantly inhibited MBM formation but not primary tumor growth or lung metastasis formation. IACS-010759 also significantly improved the survival of nude mice with IC xenografts of human melanoma cell lines with *de novo* (SKMEL5) and acquired (A375-R1) resistance to BRAF and MEK inhibitors. Together these studies implicate OXPHOS in the formation and pathogenesis of MBMs and suggest that targeting this metabolic pathway may be an effective strategy for MBM prevention and treatment. The studies also further demonstrate the significance of metabolic pathways in this disease.

Nuclear localization and stability of MITF are regulated by the bHLH-Zip domain

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Microphthalmia-associated transcription factor (MITF) is key for the establishment of the melanocytic lineage. Given its central role in melanoma and pigmentation disorders, proper characterization of the protein is required for advancing our understanding of the nature of these diseases. In this study, we have used EGFP fusions and *in vitro* mutagenesis to unravel domains in MITF-M that determine its nuclear localization and stability. We have identified three nuclear localization signals in the basic helix-loop-helix leucine zipper (bHLH-Zip) region of MITF-M, spanning residues 197-206, 214-217 and 255-265, which together orchestrate the transcription factor's nuclear localization. Importantly, variants in a number of the residues involved have been associated with Waardenburg syndrome type 2A and Tietze syndrome. Structural characterization of MITF showed that basic residues within these karyophilic signals are exposed for interactions in the absence of DNA. Moreover, neither DNA binding nor dimerization of MITF-M are required for its nuclear localization and/or retention. Our data further suggest the presence of a nuclear export signal in the N-terminal region of the protein. Finally, protein degradation assays revealed no significant changes in the half-life of MITF-M upon BRAF inhibition or mutation of S73 to alanine in BRAF(V600E) mutant melanoma cells. Interestingly, dimerization-deficient MITF-M mutants exhibited a significantly reduced stability in melanoma cells when compared to their wild type counterpart. Taken together, we have shown that in addition to its well-established role in DNA binding and dimer formation, the bHLH-Zip domain of MITF also modulates the transcription factor's subcellular localization and stability.

Melanoma cells expressing mutationally activated RAC1^{P29S} are resistant to blockade of PI3Kβ

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RAC1 mutations are the third most frequently occurring gain-of-function mutation in cutaneous melanoma, with the most frequent alteration encoding for RAC1^{P29S}. RAC1-GTP has pleiotropic regulatory functions in the cell cycle, cell-cell adhesion, motility, tumor angiogenesis, as well as invasion and metastasis. Importantly, the exact mechanism through which mutationally activated RAC1^{P29S} propagates its pro-tumorigenic effects is currently unclear. RAC1-GTP was recently shown to directly regulate the PI3'-kinase isoform PI3Kβ, leading to downstream activation of the AKT protein kinases. Here we sought to investigate whether RAC1^{P29S} propagates its oncogenic signaling through PI3Kβ in melanoma. Given the availability of PI3'-kinase isoform-selective pharmacological inhibitors, we tested whether RAC1^{P29S}-expressing melanoma cells are sensitive to blockade of PI3Kβ. Unexpectedly, RAC1^{P29S} human melanoma cells were resistant to PI3Kβ-selective blockade, suggesting that RAC1^{P29S} is not a predictive biomarker for response to PI3Kβ-targeted therapy. Furthermore, RAC1^{P29S} melanoma cell lines showed variable sensitivity to pan-class I PI3K inhibition, suggesting that PI3K is not required for melanoma maintenance in specimens that express RAC1^{P29S}. Lastly, we found that RAC1^{P29S} cell lines also showed variable sensitivity to pharmacological inhibition of PAK1 signaling, questioning the potential effectiveness of inhibitors of this pathway in RAC1^{P29S}-expressing melanoma patients. Our future research efforts will focus on the examination of other potential RAC1^{P29S} downstream effectors, such as WAVE, IQGAP1, mTORC2 and GLUT-4 as possible novel drug targets in RAC1^{P29S}-expressing melanomas.

Real-world (RW) effectiveness of first-line (1L) nivolumab (NIVO) plus ipilimumab (IPI) or NIVO monotherapy for advanced melanoma (MEL): subgroup analysis of a retrospective cohort study

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In clinical trials, NIVO alone and in combination with IPI prolongs progression-free survival (PFS) and overall survival (OS) in patients (pts) with MEL. RW data from clinical practice can help confirm these controlled-setting trials. This observational study evaluated RW outcomes and differences among key subgroups.

Pts with MEL aged ≥18 yr receiving 1L NIVO or NIVO+IPI (index date) were identified using the US Flatiron Health electronic database from Jan 2011-Jun 2017. RW outcomes in pts (treatment response, PFS, and OS) were stratified by age (≤65 vs >65 yr), *BRAF* mutation status (wild-type [WT] vs mutant [MT]), and lactate dehydrogenase (LDH) levels (≤upper limit of normal [ULN] vs >ULN). Outcomes were assessed based on in-depth patient chart review. Pts were followed until death, database discontinuation, or end of study.

Among 463 eligible pts (NIVO n=209, NIVO+IPI n=254; mean follow-up 9.2 mo), mean age was 66 yr, 44% had ECOG PS 0, 39% had LDH>ULN, 63% were *BRAF* WT, and 51% were stage M0 at index date. Overall, NIVO+IPI was associated with significantly reduced risk of progression (HR 0.65; *P*<0.001) and death (HR 0.70; *P*=0.035) compared to NIVO alone. NIVO+IPI pts had numerically higher 1 yr PFS and OS rates across key subgroups, including age, *BRAF* status, and LDH levels. After adjusting for pt characteristics, 1L NIVO+IPI showed a statistically significant reduction in risk of death for age ≤65 yr (HR 0.32; *P*=0.0001), LDH ≤ULN (HR 0.26; *P*=0.004), LDH >ULN (HR 0.18; *P*<0.0001), and *BRAF* MT (HR 0.20; *P*=0.001).

Regardless of age group, *BRAF* status, and LDH level, NIVO+IPI therapy showed superior outcomes compared to NIVO in the RW setting, with significant differences in OS for the majority of subgroups. Further study of RW safety outcomes with NIVO+IPI and NIVO is ongoing.

Grade 3/4 adverse event (AE) costs of nivolumab versus dabrafenib + trametinib as adjuvant treatment in patients with stage III *BRAF*-mutated cutaneous melanoma

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Comparative economic data between nivolumab (NIVO) and dabrafenib+trametinib (DAB+TRAM) provide valuable information to payers, patients, and healthcare providers. This study assessed the per-patient costs of all-cause grade 3/4 AEs of NIVO vs DAB+TRAM as adjuvant therapies in stage III *BRAF*-mutated (MT) cutaneous melanoma that resulted in hospitalization.
All-cause grade 3/4 AE rates of NIVO and DAB+TRAM were from the patient-level data of the CheckMate 238 trial and published results of the COMBI-AD trial, respectively. AE unit costs were from the 2015 US Healthcare Cost and Utilization Project database and inflated to 2018 USD. This analysis was under the assumption that identified patients had one type of AE that occurred once during the trial period. Due to the population difference between trials, we assumed stage IIIA patients had the same AE rates as stage IIIB/C patients in COMBI-AD. In the main analysis, costs of grade 3/4 AEs with corresponding any-grade AE reported in >10% patients receiving NIVO or DAB+TRAM were assessed. A sensitivity analysis assessed the costs for other grade 3/4 AEs.
The all-cause grade 3/4 AE costs per patient during the trial period were $517 for NIVO and $2300 for DAB+TRAM. The top three AE cost types were diarrhea ($178), hypertension ($157), and fatigue ($125) for NIVO, and hypertension ($383), pyrexia ($374), and fatigue ($346) for DAB+TRAM. After adding other grade 3/4 AEs costs in the sensitivity analysis, NIVO still had lower per-patient AE costs compared with DAB+TRAM ($2034 vs $3126).
NIVO, as an adjuvant therapy, is associated with lower average grade 3/4 AE costs that resulted from hospitalization vs DAB+TRAM for resected stage III *BRAF*-MT cutaneous melanoma. Future studies will assess the cost of a broader range of AEs and associated costs beyond the trial period.

The immunosuppressive role of Edn3/Ednrb signaling in the melanoma microenvironment

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Melanoma is one of the most immunogenic types of tumors and is also known to grow in an immunosuppressive environment, which allows immune escape and ultimately metastasis formation. Human melanoma initiation is mainly driven by activation of oncogenic protein BRaf and deletion of PTEN gene. The Endothelin Receptor b (Ednrb) signaling pathway is considered a marker for melanoma progression and in many cancer types was shown to be activated in stromal cells within the tumor microenvironment. T regulatory cells (Treg) are strong immunosuppressive cells present in the tumor microenvironment that limit tumor-specific immune responses. The relationship between endothelin signaling with immune escape and immune cell populations in the melanoma tumor microenvironment has not been defined. Using the *K5-Edn3* transgenic mouse that overexpresses endothelin 3 (Edn3) in the skin, we characterized the different populations of Ednrb$^+$ stromal cells in the melanoma tumor microenvironment. YUMM1.7 melanoma cells, which harbor BRaf$^{V600E/+}$;PTEN$^{-/-}$ mutations, as well as B16F10 melanoma cells were intradermally injected in K5-Edn3 mice and wild-type controls. YUMM1.7 and B16F10 tumors produced in *K5-Edn3* mice were significantly larger than tumors in the control mice. YUMM1.7 and B16F10 tumors were able to metastasize to the lungs only in *K5-Edn3* animals. Flow Cytometry analysis showed that among different populations of Ednrb$^+$ stromal cells, Treg cells are the only ones that are found in larger numbers in *K5-Edn3* when compared to control. These data suggest that endothelin signaling may be an important regulator of immunosuppression in the melanoma tumor microenvironment and promoter of tumor aggressiveness.

Germline Predisposition to Cancers in Melanoma Patients Presenting to Oncology

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Melanoma has been suggested to be a highly heritable cancer with high twin-twin concordance (Mucci et al., JAMA 2016). Additionally, melanoma patients have a high overall percentage (74%) of relatives with cancer (Sussman et al., SMR 2017). Patients presenting to medical oncology clinic were offered germline testing with an 81 gene panel, of which 12 genes are associated with familial melanoma. Eligibility criteria were: ≥ 2 melanomas in an individual or family; melanoma and other cancer(s) in an individual; melanoma and other cancers in at least 2 relatives, including a 1st-degree relative; age ≤35 at diagnosis; and limited family structure.

Of 130 patients, 52% were female, 96% (125) were Caucasian, 3% (4) Ashkenazi Jewish (AJ), and 1% (1) multiracial. 19% (25) were found to carry a pathogenic/likely pathogenic mutation. 40% (10) of these mutations were in genes previously associated with melanoma (*BRCA1/2, CDKN2A, BAP1, TP53*), while 60% (15) were in known cancer predisposition genes without a clear association with melanoma (*CHEK2, MSH2, PMS2, MLH1, RAD51C, BLM, MUTYH*). No AJ founder mutations were identified. The majority of mutation-positive individuals (68%, 13) did not meet any current guideline-based criteria for germline testing. Germline variant of unknown significance (VUS) rates were 48% (63) for at least one VUS and 18% (23) for multiple germline VUS. Within the mutation-positive group, 68% (17) had a family history of multiple cancers, 40% (10) had a personal history of multiple cancers, 16% (4) a personal or family history of ≥3 melanomas, and 12% (3) had limited family history.

In conclusion, melanoma patients presenting to oncology clinic with a personal or family history of cancer have a germline positive rate of 19%, which includes multiple genes not previously associated with melanoma. A relatively high rate of germline VUS also warrants further study.

Gene expression signature predicts response to first-line treatment with anti-PD-1 in melanoma

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Monoclonal antibodies targeting inhibitory immune checkpoints, such as programmed cell death-1 (PD-1), have led to a paradigm shift by achieving durable clinical responses in the treatment of multiple malignancies, including advanced stage melanoma. Although several genomic and immune predictors of response have been reported, robust biomarkers to guide treatment with these drugs in the first-line setting are still lacking. Here we used a mouse model of melanoma driven by oncogenic BRAF^{V600E} and ultraviolet radiation (UVR) to evaluate the factors that influence response to first-line treatment with anti-PD-1 drugs in controlled experimental conditions. We observed heterogeneous responses to treatment in mice bearing multiple primary tumours. Despite the increased mutation and neo-antigen load obtained by UVR exposure, these tumour features did not correlate with response, and neither did the clonality of the identified SNVs. Similarly, we did not observe a difference in responding versus non-responding tumours in terms of CD8+ T cell infiltrate, expression of PD-1 ligand 1 (PD-L1) either on tumour or antigen presenting cells, or in the expression of several components of the interferon gamma (IFNγ) pathway. We obtained concordant results in pre-treatment tumours from patients who received anti-PD-1 therapy in first-line. We developed a 26-gene signature based on differentially expressed genes in the responding vs. non-responding tumours. When applied to pre-treatment patient samples, our signature segregated patients with disease control from those who progressed. Our findings highlight the need for integrated molecular characterisation of tumours and their microenvironments to identify robust predictive biomarkers for response to immune checkpoint inhibitors.

Relevance of enrichment technique in CTC-derived explants generation

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We recently described the generation of mouse tumour xenografts from circulating tumour cells (CTC). These so-called CTC-Derived eXplants (CDX) recapitulate the cardinal histopathological features of the patient's tumour, and importantly they preserve the metastatic behaviour of the patients' disease. Thus, CDX models provide powerful tools to study mechanisms of metastasis, but their development is challenging and inefficient, so more effective protocols are needed for their production. Herein we compare CDX generation by two CTC enrichment technologies, RosetteSep^{TM} (density and negative immunoenrichment) and ClearCell® (size-based selection).
Samples from 62 metastatic melanoma patients were collected. For each patient two blood samples were taken and processed by the two approaches in parallel. Ten CDXs from nine patients were generated, a success rate of 8%. The average time to grow for the xenografts was 203 days (range 12-417). The majority of patients (7/9) for whom the CDX was successful were treatment-naïve, but all were experiencing disease progression and all had poor overall survival (average of 41.8 days). Of the 10 CDX, 3 were generated by ClearCell® and 7 by RosetteSep^{TM}. Intriguingly, ClearCell® was particularly efficient in mucosal melanomas providing a success rate in these tumours of 20% (2/8).
These preliminary data provide an, albeit inefficient, platform for CDX generation. We show intriguing bias in the two approaches, suggesting that different CTC enrichment techniques could favour specific melanoma subtypes. Further characterisation of the CDX, the original tumours and single CTC in the patients will allow us to understand the principles that are important for CDX generation. Our data also suggests that the ability to generate a CDX model for individual patients is a poor prognostic marker, reflecting the high CTC burden in late-stage disease.

Inherited Genetic Variants Associated with Amelanotic Melanoma

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Previously, in the international population-based Genes, Environment and Melanoma (GEM) Study, we found that patients with amelanotic melanoma had a poorer survival than patients with pigmented melanoma as a result of a more advanced stage at diagnosis. In GEM, 16% of melanoma deaths were a result of amelanotic melanoma although only 8% of melanomas were amelanotic. In GEM, we also found that absent back nevi, many freckles, red hair, light eyes, and decreased tannability were associated with amelanotic versus pigmented melanoma, providing information that might help identify patients at risk of amelanotic melanoma and enable earlier diagnosis. However, the underlying genetics associated with amelanotic melanoma remains largely unknown. Here, we report the results of our investigation of the associations of 47 inherited single nucleotide polymorphisms in low-penetrant melanoma-risk loci with histopathologically amelanotic versus pigmented melanoma among 2931 European origin participants in GEM. Per allele odds ratios and 95% confidence intervals were estimated using multivariable logistic regression. In models adjusted for study design features, *IRF4* rs12203592 (OR, 1.52; 95% CI, 1.22-1.88; $P < .001$) and *CCND1* rs1485993 (OR, 0.68; 95% CI, 0.55-0.84; $P < .001$) were associated with amelanotic melanoma and passed the false discovery threshold ($P = .0026$). The association of *IRF4* rs12203592 was partially attenuated after also adjusting for back nevi, freckling, eye color and tannability. The association of *CCND1* rs1485993 was independent of phenotypes. These results suggest that common, inherited variants in the *IRF4* and *CCND1* loci influence amelanotic melanoma development. The association of *IRF4* rs12203952 with amelanotic melanoma may, in part, be mediated by phenotypes, whereas the association of *CCND1* rs1485993 with amelanotic melanoma appears unrelated to patient phenotype.

EOMES+CD45RO+ effector memory T-cells determine response to combined anti-PD-1/anti-CTLA-4 immunotherapy but not to anti-PD-1 monotherapy

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Immune checkpoint blockade of the CTLA-4 and PD-1 receptors have significantly improved the survival of patients with metastatic melanoma. However, accurate predictors of response have yet to be identified. To investigate the potential mechanisms of response and resistance to immunotherapy, the transcriptomic and immunophenotypic profiles of 140 biopsies (n=104 baseline; n=36 early during treatment [EDT]) from advanced melanoma patients treated with anti-PD-1 monotherapy (n=33 responders, n=21 non-responders), or combined therapy (n=38 responders, n=13 non-responders) were characterized via RNA sequencing and multiplex immunofluorescence. Patients were classified as responders (CR/PR/SD>6 mo) or non-responders (SD≤6 mo/PD) based on RECIST. Responders to monotherapy demonstrated significantly higher expression of interferon-related and tumor-infiltrating T-cell genes (adj. P<0.05). In combined therapy, responders displayed unique expression of T-cell and NK-cell genes (EOMES, CD96, and AMICA1; adj. P<0.001). Additional CyTOF analyses revealed an increased proportion of EOMES+CD69+CD45RO+ effector T-cells at baseline in responders to combined therapy, compared to non-responders (CD4+ P=0.040; CD8+ P=0.038). These cells were also TBET^{High}, HLA-DR^{High} and CCR7^{Low}. Furthermore, this population expressed the immune checkpoint receptors PD-1 and TIGIT, and were CD57^{Low}, suggesting that they are not terminally differentiated cells. In EDT biopsies, a distinct separation in the CD8 and EOMES expression profiles was observed between responders and non-responders to combined therapy. Non-responders to both therapies demonstrated up-regulation of the hypoxic and metabolic pathways. These findings reveal potential biomarkers of response in each therapy, and highlight novel checkpoints/pathways that could be targeted to overcome resistance.

The presence of CXCR6 on CD8+ T cells in metastatic melanoma patients on pembrolizumab correlated with poor treatment outcomes.

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The advent of immune checkpoint blockers such as Pembrolizumab (anti-PD-1) has transformed melanoma treatment by blocking PD-1 on activated T cells, improving overall survival, progression free survival and overall response rates. However, some metastatic melanoma patients do not have a durable response and can present with primary or longer-term secondary resistance to pembrolizumab.

Chemokines are a family of small cytokines that induce the migration of cells through interactions with transmembrane receptors. Recently it has been shown that various types of cancer cells express chemokine receptors and the chemokines may play a role in cancer progression. CXCR6 is considered to be an inflammatory chemokine receptor, which can guide effector T cells to sites of inflammation.

The aim of this study was to develop a peripheral blood immune profile to monitor CD4^{+} and CD8^{+} T cell populations, which when combined with RECIST score, reflects the response to pembrolizumab. Whole blood was sequentially collected at every treatment cycle for up to 12 months from 16 metastatic melanoma patients receiving Pembrolizumab. The following markers were assessed by flow cytometry on a BD Biosciences Fortessa II: CD3, CD4, CD8, CD45, CD45RA, FoxP3, IL-9, HLA-DR, CD25, CD274, CD38, CXCR6 and PD-1. Average number of pembrolizumab cycles was 19 (range 2-26) at the commencement of blood collection. Patients with disease progression, confirmed by RECIST, during the 12 months of blood collection all presented with CXCR6 on CD8+ T cells. The results of this study indicate blood immune profiles can be further developed as real-time biomarkers of anti-PD1 resistance, leading to the outcome of detecting resistance and earlier consideration other treatment options for patients that do not respond to single agent anti-PD1 immunotherapy.

Clinical Application of Circulating Tumour Cells and Circulating Tumour DNA in Uveal Melanoma

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Stratification of uveal melanoma (UM) patients into groups with better or worse prognosis is based on clinicopathological and molecular features and is critical for both patient management and for directing patients towards clinical trials. However, the classification of tumours is constrained by the invasiveness of the biopsy procedure and the limited availability of tissues when enucleation is not performed. Here we evaluated the utility of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) for the management of UM.

CTCs were immunocaptured to magnetic beads, and immunostained for MART1/gp100/S100β. Low coverage whole genome sequencing (WGS) was used to determine somatic chromosomal copy number alterations (SCNA) in primary UM tumour, ctDNA and whole genome amplified CTCs. ctDNA was quantified using droplet digital PCR assay for mutations in the *GNAQ, GNA11, PLCβ4* and *CYSLTR2* genes.

In a cohort of 30 primary UM patients, CTCs were detected in 58% of patients (1-37 CTCs per 8 mL of blood), while only 26% of cases had detectable ctDNA (1.6-29 copies/mL). Neither the presence of CTCs or ctDNA were associated with tumour size or other prognostic markers. However, SCNA of CTCs showed great concordance with the enucleated primary tumour. These results support a model in which CTCs can be used to derive tumour specific SCNA relevant for prognosis. In addition, monitoring of ctDNA after treatment of the primary tumour allowed detection of progression earlier than ^{18}F-FDG-PET in two patients that develop metastatic disease during the course of the study.

The presence of CTCs in localised UM can be exploited to ascertain prognostic SCNA, while ctDNA can be used to monitor patients for early signs of metastatic disease. This study paves the way for the analysis of CTCs and ctDNA as a liquid biopsy that will assist with treatment decisions in patients with UM.

Microphthalmia-associated transcription factor regulates dynamic melanoma heterogeneity

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Dynamic heterogeneity is a prime source for drug resistance. As distinct proliferative and invasive capabilities reflect variable drug sensitivities, identifying these different responses is crucial to design effective therapies. We utilize real-time cell cycle imaging (FUCCI) in 3D *in vitro* and *in vivo* to study melanoma heterogeneity.

Mouse xenografts generated from cell lines with high microphthalmia-associated transcription factor (MITF) level displayed a homogeneous distribution of cycling cells throughout. In contrast, tumors generated from MITF-low cell lines were composed of

clusters of cycling cells and clusters of G1-arrested cells. Proliferating areas were in close proximity to blood vessels, presumably characterized by oxygen/nutrient availability. Knock-down of MITF in MITF-high melanoma cells resulted in the same clustered phenotype presented in xenografts generated from MITF-low melanoma cells. Melanoma spheroids recapitulated the *in vivo* cycling behavior. MITF was undetectable within the hypoxic G1-arrested spheroid core, indicating hypoxia-induced downregulation. Modulation of MITF expression impacted spheroid architecture and size, with overexpression giving rise to less compacted structures and *vice versa*. MITF protected from cell cycle arrest induced by oxygen/nutrient deprivation. High MITF levels prevented cell cycle arrest by reducing the cell-intrinsic propensity to arrest in response to low oxygen/nutrient and concurrently by allowing sufficient supply of oxygen/nutrients to cells. The latter is achieved through decreased cell-cell adhesion resulting in the generation of looser, 'spongier' tumors that may allow more efficient oxygen/nutrient diffusion.
Taken together, MITF regulates dynamic heterogeneity, which in turn impacts on drug sensitivity.

RAB27A promotes melanoma cell invasion and metastasis via regulation of pro-invasive exosomes

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Despite recent advances in targeted and immune-based therapies, advanced stage melanoma remains a clinical challenge with a poor prognosis. Understanding the genes and cellular processes that drive progression and metastasis is critical for identifying new therapeutic strategies. Here, we found that the GTPase RAB27A was overexpressed in a subset of melanomas, which correlated with poor patient survival. Loss of RAB27A expression in melanoma cell lines inhibited 3D spheroid invasion and cell motility *in vitro*, and spontaneous metastasis *in vivo*. The reduced invasion phenotype was rescued by RAB27A-replete exosomes, indicating that exosomes drive RAB27A-mediated invasion. Furthermore, while RAB27A loss did not alter the number of exosomes secreted, it did change exosome morphology and the abundance of exosomal proteins associated with cancer cell migration and metastasis. These findings support RAB27A as a key cancer regulator, as well as a potential prognostic marker and therapeutic target in melanoma.

Socioeconomic factors and outcome in melanoma

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The association of deprivation with increased Breslow thickness and poorer melanoma prognosis has been reported. In this study, variables of interest were factors that had a known link to deprivation: smoking, body mass index (BMI), serum 25-hydroxyvitamin D_2/D_3 level (vitamin D), psychosocial stress and perceived control over health. 2183 patients from the Leeds Melanoma Cohort were included and median follow-up was 6.7 years.

Linear regression was used to assess predictors of Breslow thickness. 100x natural log was taken so that coefficients were interpreted as a percentage change. Smoking at diagnosis was independently associated with increased Breslow thickness (12.02%, CI 1.03-23.01, p=0.03) as was BMI >35 (17.66%, CI 2.64-32.67, p=0.02) and a deficient vitamin D level (<20 nmol/L) (23.28%, CI 8.88 to 37.68, p=0.002) in a multivariable model also including age, sex, tumour site, and deprivation score. Smoking and vitamin D were also associated with increased risk of melanoma death (smoking HR 1.53, CI 1.04-1.88, p=0.01, deficient vitamin D HR 1.54, CI 1.02 to 2.32, p=0.04) in a multivariable model but BMI was not. Exposure to life stressors, such as financial strain, was not associated with either Breslow thickness or melanoma death in multivariable analysis. The Townsend Material Deprivation Score also showed no association with death from melanoma. A high 'chance' perceived health control, where the individual feels that chance controls their health outcomes, was associated with increased melanoma death (HR 1.35, CI 1.06 - 1.71, p=0.03) in a multivariable model.

The study gives evidence that it is not deprivation per se that is responsible for the known association between deprived groups and poorer outcomes but other contributing factors. The association of health locus of control with survival needs confirmation and further exploration but suggests a role for improving health empowerment to reduce melanoma death.

CADM1 is a TWIST1-regulated suppressor of invasion and survival

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Metastatic melanoma is the deadliest form of skin cancer; however, patients diagnosed prior to metastatic dissemination have a good prognosis. The transcription factor, TWIST1 has been implicated in enhancing the migration and invasion steps within the metastatic cascade, but the range of TWIST1-regulated targets is poorly described. In this study, we performed expression profiling to identify the TWIST1-regulated transcriptome of melanoma cells. Gene ontology pathway analysis revealed that TWIST1 and epithelial to mesenchymal transition (EMT) were inversely correlated with levels of cell adhesion molecule 1 (CADM1). Chromatin

immunoprecipitation (ChIP) studies and promoter assays demonstrated that TWIST1 physically interacts with the CADM1 promoter, suggesting TWIST1 directly represses CADM1 levels. Increased expression of CADM1 resulted in significant inhibition of motility and invasiveness of melanoma cells. In addition, elevated CADM1 elicited caspase-independent cell death in non-adherent conditions. Expression array analysis suggests that CADM1 directed non-adherent cell death is associated with loss of mitochondrial membrane potential and subsequent failure of oxidative phosphorylation pathways. Importantly, tissue microarray analysis and clinical data from TCGA indicate that CADM1 expression is inversely associated with melanoma progression and positively correlated with better overall survival in patients. Together, these data suggest that CADM1 exerts tumor suppressive functions in melanoma by reducing invasive potential and may be considered a biomarker for favorable prognosis

Molecular factors modulating vitamin D response in BRAF^{V600E}-containing melanoma cells

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The aim of this study was to determine how the anti-cancer effects of vitamin D may be 'rescuable' in the context of metastatic melanoma. We explored the possibility that mediators of the epithelial mesenchymal transition (EMT), such as SNAIL1, may repress the function of vitamin D receptor (VDR) thus further contributing to tumor progression. We proposed that by inhibiting SNAIL1 function/expression, VDR activity could be restored leading to enhanced expression of E-cadherin, an important effect of VDR for limiting tumor invasiveness and spread. We confirm that the A375 and SKMEL28 melanoma cell lines express appreciable levels of functional VDR that is capable of transactivation through exposure to the active vitamin D cognate ligand (1,25D). In contrast, 1,25D treatment failed to inhibit Wnt pathway signaling and proliferation of both cell lines. Expression analysis of downstream VDR target genes indicate an impaired response to 1,25D, with the noted exception of CYP24A1 that mediates catabolism of 1,25D. Importantly, 1,25D exposure increased the expression of E-cadherin within SKMEL28, which harbors significantly reduced levels of SNAIL1 compared to A375 in which E-cadherin expression is negligible. siSNAI1 knockdown in A375 was insufficient to restore a response to 1,25D nor rescue E-cadherin expression. We speculated that a further contributory factor for an impaired response to 1,25D could be due to the presence of the BRAF^{V600E}, inducing constitutive kinase activity. Application of the BRAF^{V600E} specific inhibitor PLX4032, we observe decreased expression of SNAIL1 within A375, however unexpectedly VDR mRNA and protein was also diminished, indicating a dependency of VDR expression upon the MAPK/ERK pathway. Overall, our data suggest a complex set of co-repressor and post-translational processes that modulate VDR response in metastatic melanoma.

The International Melanoma Database and Discovery Platform (IMDDP): progress to date and next steps

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Large, real-world, international datasets are necessary to develop robust and broadly generalizable predictive and prognostic melanoma patient (pt) outcome models that ultimately inform clinical decision-making. The initial (Phase I) objective of the IMDDP, conceived and developed in 2015, was to inform updates to the American Joint Committee on Cancer (AJCC) melanoma staging system.

Using the contemporary IMDDP as the analytic backbone, the AJCC Melanoma Expert Panel evaluated melanoma-specific survival of Stages I-III cutaneous melanoma pts to explore new prognostic factors in staging. Towards this end, we launched a communication campaign to engage collaborators, coordinated DUAs with participating institutions, developed an analytic plan and distributed a detailed data dictionary, received, quality-assured, and merged all datasets into an analytic database comprised of data for >46,000 pts from 10 institutions worldwide, conducted statistical analyses, and interpreted and shared results. A successful team-based analytic effort informed the AJCC 8th edition staging system that was published in 2017 and formally implemented January 2018.

Leveraging this success, we are expanding IMDDP to address contemporary issues in melanoma prognostication, and clinical tool development and validation. For this Phase II effort, we expanded inclusion criteria (Stage I-IV pts), # of covariates requested (21 in Phase I to 55), and # of contributing institutions (10 in Phase I to 25). We also conducted a detailed database survey (N= 30 institutions) to inform a consistent approach for collection and coding of new covariates. During Phase II, we plan to evaluate conditional and relapse-free survival, validate the 8th edition staging system, and develop and validate clinical tools.

We continue to extend an invitation to collaborate to all qualifying institutions worldwide.

Enhancing melanoma clinical data extraction in the era of big data using Natural Language Processing

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Medical records contain many informative data elements that are valuable for clinical research. Unfortunately, most such data points are stored in unstructured documents, particularly in cases involving legacy data, and require manual data extraction into a structured format to render the information readily accessible, searchable, and analysis-ready. Manual data extraction is labor intensive and

oftentimes cost-prohibitive, particularly when dealing with large cohorts of patients.

To establish a higher-throughput methodology for data extraction, we developed a novel framework using natural language processing (NLP) and a custom decision-rules algorithm to extract, transform and load melanoma primary pathology features from pathology reports into a structured database. We also developed a novel scoring system to assess the confidence level of records generated by the algorithm to obviate manual review of high confidence records and flag low-confidence records for targeted manual review.

The algorithm produced 368,624 individual melanoma primary prognostic factors, characterizing primaries for 23,039 patients. From these, a subset of 147,872 prognostic factors were compared against an existing, manually extracted dataset. Overall, an exact match was noted in 90.4% of all data points compared between datasets. Our confidence scoring algorithm correctly flagged NLP-compiled records as either high or low confidence in 96.3% of cases.

The principles used in the development of our algorithm could potentially be expanded to include other melanoma disease characterization data points in semi-structured templates, such as mutation data, other laboratory results, and other disease events. This NLP platform can identify and extract melanoma primary prognostic factors with an accuracy comparable to manual extraction, with much greater efficiency.

Catch me if you can! -Novel immune escape mechanisms in melanoma metastases of the brain-

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The immune system is one of the key modulator directing tumor growth and progression, but also therapy responsiveness. Recent reports clearly idicate that drug activity in the brain is not only directed by the drug susceptibility to penetrate the blood-brain-barrier, but is most likely modulated by the cellular composition of the brain-specific metastatic niche which modulates establishment and outgrowth of brain metastases. The immune microenvironment in brain metastases is active with a high density of tumor-infiltrating lymphocytes in certain patients and, therefore, may serve as a potential treatment target.
To study the reciprocal communication between tumor cells and the immunoactive infiltrate in the brain but also its impact on therapy efficacy, we were able to establish new preclinical models which allow for the first time to study homing specific mechanisms of brain-seeking melanoma cells in the presence of an intact immune system. By using our novel models, we were able to identify unknown immune escape mechanism of melanoma cells penetrating the brain.

Identification of inherited factors for melanoma susceptibility

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Cutaneous malignant melanoma is the most aggressive form of skin cancer with an annual incidence increase of 5% in the Swedish population. Early diagnosed tumors have a far more favorable prognosis compared to disseminated melanoma. Thus, with earlier diagnosis more lives could be saved. For this purpose, there is a need to develop successful strategies to identify individuals at greatest risk. The aim of this project is to identify novel melanoma susceptibility genes to facilitate prevention and early detection of melanoma. Around 10% of all melanoma cases are considered hereditary. The causative gene is to date known in about 20% of the Swedish melanoma families. Knowledge about inherited mutations would enable us to identify and monitor risk individuals. To search for novel melanoma genes, we have executed next-generation sequencing of high-risk melanoma patients with unknown genetic background. So far, we have performed exome sequencing on 15 Swedish melanoma families. Besides findings of known cancer susceptibility genes like BAP1 and BRIP1, we have detected novel melanoma candidate genes suggested to be involved in many essential cellular processes like autophagy, vesicle transportation and cell proliferation. We want to investigate the biological significance of observed loss-of-function variants of these candidate genes and determine its importance in melanoma susceptibility, for example by creating knock-down cells using CRISPR-Cas technology to analyze cellular down-stream effects when a specific gene is silenced. We will also screen additional melanoma patients for mutations in these candidate genes. We believe that the result from these studies will make a substantial contribution in understanding the genetic basis of melanoma susceptibility and lead to more individualized management of melanoma families with specific high-risk mutations.

A multidimensional comparative analysis of genetically engineered mouse model of melanoma with syngeneic transplant models

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Background

Genetically engineered mouse models (GEMMs) of cancer, hold the promise as autochthon models of human malignancies. However, the application of GEMMs in preclinical testing remains limited, due to multiple factors but chiefly due to the difficulties to generate larger cohorts of animals as well as to heterogeneity of tumors. Syngeneic transplanted models can provide an alternative to GEMMs. In syngeneic models, cells of a cell line derived from a murine tumor are implanted in a murine host, either orthotopically or by subcutaneous injection. We looked at how melanoma tumors of a GEMM model of melanoma, iBIP2, compare to transplanted tumors of cell lines derived from the same iBIP2 GEMM model.

Objective:

To compare at single-cell resolution melanoma tumors from a GEMM model, iBIP2, with syngeneic transplanted tumors derived from the iBIP2 tumors.

Methods:

We analyzed melanoma cells and cells of the tumor microenvironment using single-cell RNA sequencing, mass cytometry as well as bulk proteomic, qPCR and IHC.

Results:

The transplanted tumors show major differences compare to the GEMM tumors, notably: reduced TME with, reduced myeloid compartment, lack of T cells as well as reduced non-immune stroma. Previously described heterogeneity of melanoma cells in human tumors (MITF^{hi} vs. AXL^{hi}) is maintained in GEMMs but lost in the transplanted models. As a consequence, signaling between melanoma cells and cells of the tumor microenvironment is perturbed. This however, does not impact on drug sensitivities as both the GEMM model, iBIP2, and transplant tumors are insensitive to PD1 inhibitors.

Conclusion: GEMM and syngeneic tumors are not interchangeable for preclinical studies of immune therapies. GEMMs maintain a more realistic representation of intratumoral heterogeneity of human tumors.

RSK Regulates Metabolic homeostasis in Melanoma

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Metabolic reprogramming is a hallmark of cancer that includes increased glucose uptake and accelerated aerobic glycolysis. This phenotype is required to fulfill anabolic demands associated with aberrant cell proliferation and is often mediated by oncogenic drivers such as activated BRAF. In this study, we show that the MAPK-activated p90 ribosomal S6 kinase (RSK), a family of MAPK-activated protein serine/threonine kinases frequently deregulated in several types of cancer, is necessary to maintain glycolytic metabolism in BRAF-mutated melanoma cells. Indeed, a phosphoproteomic study performed in the lab identified PFK2, an enzyme responsible for regulating the rates of glycolysis, as a potential RSK substrate. Using pharmacological inhibitors and in vitro experiments, we confirmed that RSK phosphorylates PFK2 on Ser466 and Ser483 in multiple cell types, including BRAF-mutated melanoma cells. We also found that RSK-mediated phosphorylation of PFK2 within its regulatory domain modulates its activity. Consistent with this, RSK inhibition reduces lactate production as well as the TCA cycle, suggesting a role for RSK in increasing glycolytic flux. We also found that expression of a phosphorylation-deficient mutant of PFK2 decreases lactate production in melanoma cells. Moreover, we found that RSK-mediated phosphorylation of PFK2 is required for melanoma growth in nude mice. Taken together, we show that metabolic rewiring in melanoma requires RSK-mediated PFK2 phosphorylation and activation. These results suggest that RSK is necessary for melanoma cell proliferation and represents an attractive new target for therapeutic intervention.

Serum DCLK1 levels predict response and survival in melanoma

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Doublecortin-like kinase 1 (DCLK1) is reported to identify tumor stem cells in the intestine, and elevated DCLK1 has been correlated with EMT phenotype and poor prognosis in colorectal cancer, pancreatic cancer, and renal cancer. We evaluated DCLK1 levels in serum and tissue of melanoma patients. ELISA and Western blot were performed on serum before and where possible after treatment with either targeted agents or immune checkpoint inhibitors. IHC analysis was performed on the same patients' tumor tissues.

Additionally, analysis of DCLK1 and correlative gene expression profiles was performed using TCGA Melanoma dataset. The intensity of DCLK1 staining in melanoma tumor tissues is increased compared to normal tissues. DCLK1 levels in the serum were elevated in melanoma patients (n=20) compared to healthy volunteers. Next, we compared DCLK1 levels in 10 patients for whom pre and post treatment samples were available. Seven had elevated DCLK1 at baseline and had a significant decrease after treatment while three patients had no drop. The patients with a decrease in DCLK1 post-treatment demonstrated radiologic response as well, while the patients with no change showed either no improvement or progression (p<0.002) radiologically. These data suggest that reduction in serum DCLK1 levels following therapy for melanoma was associated with clinical response, and the absence of change in DCLK1 after therapy was associated with poor response. Preliminary analysis of baseline DCLK1 serum levels in the entire population studied indicated survival differences as well. Analysis of the TCGA dataset demonstrated that melanoma patients with high expression of DCLK1 had worse overall survival (p<0.05) compared to patients with low levels, suggesting that DCLK1 RNA levels could be used as a prognostic biomarker. Together, these data suggest potential utility of DCLK1 as a prognostic and on-treatment biomarker for melanoma.

Clinical Experience with Acral Melanoma at the University of Southern California

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Background: Acral melanoma (AM) occurs on the palms, soles and nailbeds, with higher prevalence among darker skin pigmented populations. Molecular studies of AM reveal low rates of single nucleotide variants but increased copy number and structural variants. Because of its distinct clinicopathological behavior, improved interventions specific to AM are needed. We report our institutional experience treating AM at the University of Southern California.
Methods: We retrospectively reviewed all patients with melanoma located on acral sites from 2007-2017. Demographic and clinical variables were obtained from medical records and investigated in relationship with patient outcomes. Among patients receiving systemic therapy, tumor responses were evaluated by RECIST 1.1, and Kaplan-Meier curves were constructed to estimate survival.
Results: Among 292 melanoma cases reviewed, 71 met inclusion criteria for AM. The median age was 58.5 (range 26-97), and the majority were of Hispanic descent (77.5%). The predominant histology was acral lentiginous (86.4%). The majority of AM were located on the lower extremity (83.3%), either plantar or subungual. BRAF V600 mutations were infrequent (8%), while NRAS (29%) and KIT (26.7%) alterations occurred more often. Among 20 patients treated with systemic therapy, the ORR was modest for cytotoxic therapy (15.4%) and checkpoint inhibitors (10.5%), but higher for targeted therapy (66.7%), including 2 complete responses. There was 1 partial response to TVEC (1/3) but no responses with high dose IL-2 (0/3). Median OS for patients receiving systemic therapy was 11.6 months; median PFS was 36.3 months.
Conclusion: When eligible, AM patients appear to derive greater benefit from targeted therapy, compared to cytotoxic therapy or checkpoint inhibition. Further investigation is needed to identify additional biomarkers for targeted therapy in this rare melanoma subtype.

Integration of multi-omics data and a novel melanoma-specific gene panel to support therapy decisions in rare melanomas

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Immune checkpoint inhibitors are currently the only therapy option with potential long-term benefit for BRAF/NRAS/CKIT (triple) wildtype (wt) melanomas due to lack of driver mutations with approved targeted therapies. This group of melanomas includes cutaneous melanomas and rare melanoma subtypes such as mucosal melanoma (incidence 2.7/million) and ocular melanoma (incidence 5-8/million), which is known to have limited response to immunotherapy.
To support therapy decisions in triple wildtype melanomas, we set up a multi-omics analysis approach whereby a metastasis on progression is analysed via whole exome, whole genome and RNA sequencing. For gene expression comparison, we used the TCGA cutaneous or uveal melanoma cohort to identify active oncogenic pathways. Furthermore, for faster and economic analysis, we developed and employed a melanoma-specific custom gene panel, the "MelArray", to calculate tumor mutational burden and identify SNVs and CNVs in 190 genes relevant for melanoma progression and treatment.
We comprehensively analysed a cohort of triple wt tumors from 10 melanoma patients. All patients presented with a low mutational burden and multiple actionable variants, matching off-label therapies and clinical trials. The MelArray panel was able to identify all actionable variants detected in whole exome sequencing when present on the panel and additional potentially actionable variants. RNAseq analysis not only verified genetic variants but also led to identification of targetable cMET and HGF overexpression in a young patient with an aggressive course of disease who was subsequently treated off-label with the cMET inhibitor crizotinib. Our approach demonstrates the effective translation of high-dimensional and expensive technologies into a clinically relevant, cost effective, and rapid assay for clinical decision support.

Biospecimen Banking In The Neo-adjuvant Setting

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Melanoma Institute Australia has run 3 neo-adjuvant studies for stage III cutaneous melanoma, with over 70 patients treated thus far. These studies involve procuring a biopsy before systemic therapy (PRE), a biopsy early during treatment (EDT) and a dissection after a set period of therapy. This approach complemented our existing TEAM (Treat Excise Analyse Melanoma) protocol where we had previously encountered resected tumours with no residual melanoma. We also collected bloods at set time-points for biomarker studies under our liquid biopsy program.

Expecting a complete or near-complete response to treatment (either radiological or pathological) we modified our banking protocol and instructed clinical trials staff, medical oncologists, radiologists, surgeons and the pathology departments. The PRE and EDT biopsies were often needle core biopsies performed by radiologists. Five to 7 cores were requested to ensure tissue for snap freezing as well as formalin fixation.

Where possible, the pathology staff processing the surgical specimen were given recent imaging reports that showed the radiological response to treatment and the anatomical tumour location. In some cases the location is difficult to assess if the tumour has regressed and we plan to mark the tumour location at PRE (e.g. with a metal clip insert by the radiologist) for future studies. For surgical dissection specimens, we bank fresh frozen tissue, and collect tissue for tumour dissociates and cell lines, Pathology cut-up staff are alerted that there may not be tumour in these samples so tissue is not wasted. Pathologists and pathology registrars new to neo-adjuvant banking receive training and supervision from more experienced staff.

Pathology reports of the surgical excision document the degree and type of response to therapy, pattern of regression and TILs infiltrate, which allows clinicians to give patients detailed feedback as to response and prognosis.

RICTOR effects on melanoma cell proliferation and invasion through MITF regulation

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Cutaneous melanoma is characterized by frequent mutational activation of the MAPK and PI3K signaling pathways, both playing a central role in cell survival and proliferation. Despite considerable efforts to specifically target these mutations, drug resistance occurs in most cases. Regarding PI3K or dual PI3K/mTOR inhibitors, this resistance is due to AKT reactivation linked to a feedback mechanism involving the mTORC2 complex and in particular its scaffold protein RICTOR. Our team has previously shown that the RICTOR locus is amplified in melanoma cells and that RICTOR overexpression in melanocytes induces cell proliferation and PI3K pathway activation. This study aims to decipher RICTOR implication in melanoma development. RICTOR was overexpressed in melanoma cell lines and its effects on clonogenicity, 3D growth and PIK3 and MAPK pathways activation were evaluated. We showed that RICTOR overexpression inhibits melanoma cell proliferation, but interestingly activates PI3K and MAPK pathway through increased phosphorylation of AKT (Ser 473) and ERK. Yet, RICTOR overexpression stimulates cell proliferation in melanoma spheroids culture. Furthermore, an increase in melanoma cell invasion was observed in RICTOR transfected cells. Several studies have highlighted the role of MITF in the switch between proliferation and invasion in melanoma cells. We showed that RICTOR overexpression decreases MITF expression in melanoma cells and reciprocally, RICTOR knockdown increases MITF expression. Our findings suggest a key role of RICTOR in melanoma development through MITF regulation, impacting on melanoma cell proliferation and invasiveness. Experiments are in progress to investigate cancer stem cell-like characteristics in RICTOR overexpressing cells.

Phosphoproteomic profile of melanoma resistant to combined PI3K/ERK inhibition

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Background: Drug-resistant melanoma is very difficult to treat, and a novel approach to overcome resistance is needed. The present study aims at identifying the alternate pathways utilized by the drug resistant melanoma cells for their survival and proliferation and further to use the multiple signaling pathway inhibitors for radiation sensitization.

Materials and Methods: The drug-resistant melanoma cells (B16F10R) were established by treating the cells with a combination of

U0126 (ERK1/2 inhibitor) and LY294002 (PI3K-AKT kinase inhibitor) in a dose-escalating manner.

Result: Using high resolution Orbitrap mass spectrometer, we identified 363 altered proteins of which, 126 hyperphosphorylated and 137 hypophosphorylated (1.5-fold change) in B16F10R cells compared to the parental cell line (B16F10C). Data suggested that multiple RNA splicing-regulatory proteins and cell cycle proteins may play a role in maintaining its drug resistant state. Histone deacetylases 2 (HDAC2), Src protein-tyrosine kinase (Src) and Structural maintenance of chromosome 3 protein (SMC) among others, are key proteins responsible for drug resistant melanoma. B16F10R cells also showed cross resistance to SP600125 (JNK inhibitor) and LDN193189 (BMP inhibitor).

Conclusion: Our study highlights possible role of spliceosome and cell cycle regulators in development of drug resistance in melanoma. Therefore, designing leads for targeting them along with key signaling pathways may be helpful in treatment.

ASSESSMENT OF QUALITY OF LIFE IN PATIENTS WITH METASTATIC MELANOMA IN REAL CLINICAL PRACTICE IN FRANCE

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Significant advances were recently observed in the treatment (trt) of metastatic melanoma (MM). With 50% of patients (pts) now reaching a 2nd line of trt and a significant improvement in survival, the assessment of quality of life (QoL) during whole disease is necessary. The objective of this work is to evaluate the impact of the disease progression and of the 2nd line on the QoL evolution in real clinical practice.

QoL is collected through MelBase, a prospective French multicentric cohort dedicated to the follow-up of adults with MM. QoL is assessed using the EQ-5D and the FACT-M questionnaires, at inclusion, every 3 months and at each trt change, until death. To assess longitudinal changes from baseline to the event (progression or 2nd line of trt), mixed-effect models for repeated measures analysis were used that controlled for baseline covariates.

QoL is assessed on 1435 pts included between 2013 and 2018. Median follow-up is 9.4 months and 613 pts died during follow-up. From models, in 1st trt line, the mean utility score is 0.830 [CI$_{95\%}$:0,818-0,843] and 129.46 [128.02;130.90] for FACT-M. In progression free survival (PFS) state, the mean utility score is 0,832 [0,820-0,843] and 129,36 [128,54-131,34] for FACT-M. At the time of line change, there is a decrease of 0.027 [-0.03;-0.02] for utility, 1.82 [-1.88;-1.76] for the FACT-G and of 2.98 [-3.05;-2.91] for FACT-M compared to the 1st trt line. At the time of progression, there is a decrease of 0.034 [-0.04;-0.03] for utility, of 3.09 [-3.15;-3.05] for FACT-G and of 4.58 [-4.65;-4.51] for FACT-M compared to PFS.

Based on filled in questionnaires, in Melbase cohort, MM pts's QoL seems to be fairly stable during the 1st trt line and PFS state. Similar trends are obtained in 2nd line of trt and after progression. A drop is observed at the time of the event.

Dabrafenib and trametinib combination versus other interventions as adjuvant therapy for advanced cutaneous melanoma: a network meta-analysis

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Given the absence of head-to-head trials, we sought to perform a network meta-analysis (NMA) of dabrafenib and trametinib (D+T) versus nivolumab (NIV), pembrolizumab (PEM), ipilimumab (IPI), vemurafenib (VEM), chemotherapy (CHE), and interferons (IFNs) as adjuvant therapy in completely resected, high-risk cutaneous melanoma patients.

A systematic literature review of bibliographic databases, clinical trial registries, and conferences was performed. Outcomes of interest included overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS), grade 3/4 adverse events (AEs), serious AEs (SAEs), and discontinuations due to AEs (DAEs). RFS was also assessed for studies that reported results on BRAF+ patients. All outcomes were synthesized using Bayesian NMA.

Constant hazard ratio (HR) NMA demonstrated D+T had significant reductions in RFS and DMFS compared to IPI, IFNs and CHE; the risk was comparable with respect to NIV, PEM (RFS only; DMFS data not available) and VEM. This was consistent when

restricted to studies that reported RFS on BRAF+ patients. Constant HR NMA showed that D+T had significantly better OS compared to placebo and IFNs (except high dose IFN alpha-2b) and was also comparable to IPI and CHE. OS was not available for NIV and PEM.

D+T had significantly fewer DAEs compared to intermediate dose IFN alpha-2b and PEG-IFN alpha-2b, significantly more than NIV, and was comparable to others. For grade 3/4 AEs, D+T had significantly less AEs compared to VEM and significantly more compared to PEM. For SAEs, D+T was comparable to IPI and significantly higher compared to NIV.

D+T is a new treatment option for adjuvant melanoma, with improved efficacy over historical options (IPI, IFN) and comparable efficacy (RFS) over available follow-up to newer treatment options (NIV and PEM).

A systems biology approach to studying melanoma invasion in a 3D tumor microenvironment

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Late-stage melanoma is characterized by increased tumor invasion and poorer patient outcomes. Various studies have detailed changes in the melanoma cells during invasion as well as the effect of tumor microenvironment. At the same time, there is increasing evidence that physical characteristics of the extracellular tumor microenvironment such as matrix stiffness and contractility play an important role in determining cell motility. Recent work characterizing extracellular matrix (ECM) in melanoma patients demonstrated changes in both skin and lymphatic tissue architecture that could affect melanoma invasion. Based on these observations, we hypothesized that tumor cells encounter different tissue architecture as they migrate systemically and this tissue-tumor interaction might influence metastatic lesion. We approached this question by using fibroblasts isolated from multiple tissues to recreate ECM *in vitro* that closely resembles an *in vivo* ECM. We observed that migration rate of melanoma cells is affected by the tissue they encounter, which also concurs with the observed rates of metastatic lesions in melanoma patients. We are now characterizing the migrating melanoma cells at single cell resolution to identify non-genetic changes that may occur in melanoma cells exposed to different tissues. Together, the experiments described here will elucidate the role(s) of extracellular matrix proteins in modulating systemic melanoma invasion in the patients. Our work has important implications towards the development of rational therapies for metastatic melanomas.

PD-L1 expression on pre-treatment circulating tumour cells, but not serum VEGF, is predictive of melanoma response to pembrolizumab

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Immune checkpoint inhibitors including pembrolizumab and nivolumab have revolutionised treatment of melanoma with a small proportion of patients deriving durable disease control lasting up to 5 years. However, majority of patients do not respond to these drugs that are costly and can lead to substantial toxicity. Therefore, there is an urgent need for biomarkers that can identify patients that will respond to these therapies.
We therefore used multi-parametric flow cytometry to identify circulating tumour cell (CTC) subpopulations based on the expression of melanoma markers MCAM, MCSP, ABCB5, CD271 and RANK in metastatic melanoma patients prior to commencing treatment with pembrolizumab (n=40) or with ipilimumab alone or in combination with nivolumab (n=14). In particular, we evaluated the expression of PD-L1 on CTCs in relation with response to treatment and progression free survival (PFS). Serum vascular endothelial growth factor (VEGF) concentrations were also evaluated.
Pre-treatment serum VEGF concentrations were significantly higher in patients not responding to ipilimumab treatment (alone or in combination with nivolumab) (p=0.0094). In contrast, serum VEGF was not predictive of response to pembrolizumab. Pre-treatment CTC positivity was not associated with response or PFS in either cohorts. However, PD-L1 expression on CTCs was associated with response to therapy. PD-L1 expression was found in 13 of 16 responders with detectable CTCs, while only 4 of 10 non-responders had PD-L1 detectable on their CTCs (p=0.0425). Expression of PD-L1 on CTCs was also associated with longer PFS (p=0.0117).
Our results provide evidence for the first time in melanoma, that detection of PD-L1 on CTCs is predictive of response to pembrolizumab and longer PFS.

Comparison of real-world outcomes pre- and post-immuno-oncology (IO) approval: an observational cohort study (OPTIMIzE) in advanced melanoma

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With the treatment landscape rapidly evolving for patients with melanoma, there is a need for evidence on the real-world use and outcomes for those receiving IO therapies. OPTIMIzE (NCT02780089) is an observational, US-based multisite study of adult patients with unresectable or metastatic melanoma consisting of a prospective cohort receiving first-line (1L) IO treatment (IO: 2011-2018 Q2; n=244) and a retrospective cohort receiving treatment before ipilimumab approval (pre-IO; 2007-2011 with $\geq$1 y follow-up; n=71). The most common pre-IO 1L therapy was temozolomide (49%). 60% of IO patients received anti-PD-1 monotherapy (nivolumab, 13%; pembrolizumab, 47%), 33% received nivolumab + ipilimumab, and 7% received ipilimumab alone. Compared with pre-IO patients, IO patients were older (mean age 59.2 vs 64.5 y, P=0.003) and treated more in academic settings (16% vs 39%, P<0.001), and had a higher comorbidity burden (65% vs 80%, P=0.008) and higher rates of LDH testing (46% vs 77%, P<0.001). Average (mo [range]) therapy duration showed significant differences between pre-IO and IO (3.7 [0.0-52.1] vs 4.4 [0.0-25.2], P=0.026). No significant differences were seen in grade 2 (38% vs 46%, P=0.240) or grade 3-4 adverse events (24% vs 32%, P=0.174). IO patients had significantly higher objective response and disease control rates (15% vs 39%, P=0.003; 38% vs 63%, P=0.003) and 1 y survival probability (34% [95% CI: 23%, 45%] vs 73% [95% CI; 66%, 78%]). Adjusting for baseline differences, IO patients had a 74% lower likelihood of death (HR 0.26 [95% CI; 0.17, 0.39], P<0.0001). In real-world clinical practice, patients with melanoma receiving IO had superior overall survival compared with pre-IO patients, validating the improved outcomes in the real world with those observed in clinical trials.

Melanoma Associated Fibroblasts as Possible Effectors in Microenvironment of Melanoma

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The tumor microenvironment plays an essential role in the biology of tumors, which is already well documented in different types of tumors. Cancer-associated fibroblasts (CAFs) are composed of the tumor stroma.
We isolated the melanoma-associated fibroblasts (MAFs) from skin metastasis of melanoma, and we studied the biological effect to the melanoma lines with the aim to monitor expression of differentiation markers in melanoma lines. On the other hand, MAFs can also induce the expression of keratin 19, 14 and 8 in normal keratinocytes in vitro co-cultured model. We also demonstrated the influence of MAFs from melanoma in long-term culture with 3T3 fibroblasts. MAFs can induce expression of multipotent stem cells markers such as Oct4 and Nanog in 3T3 fibroblasts. MAFs are also able to induce expression stem cells markers Oct4, Nanog, CD271 in melanoma cell line in non-adhesive condition. In culture, we also studied the possible role of MAF in mechanisms of resistance melanoma to target therapy by B-raf inhibitors.
We demonstrated the biological function of MAFs and their possible influence on the expression of melanocytic markers in melanoma lines and also their effect on the normal keratinocytes. We also demonstrated the biological effect of MAFs to induction of stem cells markers in melanoma cells.
The biological effect of MAFs is very similar as the effect of CAFs in different types of tumors, and their role in cancer progression despite melanoma may be crucial to keep the tumor microenvironment, and their possible role in melanoma resistant to target therapy is accepted.

Possible abscopal effect induced by cryotherapy in a patient with metastatic skin melanoma that progressed on treatment with anti-CTLA-4

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Immunotherapy approaches using anti-CTLA-4 antibodies represent an integral part of the treatment in a patient with advanced melanoma since 2010. Effect of immunotherapy is only in some patients with advanced melanoma, therefore looking for ways to help immune cells to recognize tumor antigens. Radiotherapy is one of the ways to increase the number of tumor antigens that are released into the environment and can be absorbed by Antigen Presenting Cells (APCs) and subjected to T lymphocytes.

We present patient treated with anti-CTLA-4 therapy for recurrent metastatic melanoma in the scar on the back after primary melanoma Breslow 1.5 mm diagnosed in 2009. Disease progression was presented as skin and subcutaneous metastasis in the original scar. CT detected disease progression as metastatic axillary lymph nodes and liver metastasis two months after anti-CTLA-4 therapy. We used palliative cryotherapy of the skin metastases to reduce the tumor burden partially two months after anti-CTLA-4 therapy. The effect of cryotherapy was seen not only on the skin and subcutaneous metastases on the back, but there was also the disappearance of axillary lymph nodes and liver metastasis two months after cryotherapy. Activation of the immune system response after cryotherapy suggests decreasing of lactate dehydrogenase (LDH) and S100 protein to normal laboratory values. Although there are numbers of preclinical data that indicate the potential synergy effect of cryotherapy and activation of the immune system. Cryotherapy applied to the skin metastases have stimulated increase disintegration of tumor antigens with activation of the immune system and with the systemic (abscopal) effect on the lymph nodes and liver metastasis, although the patient has disease progression after prior treatment with anti-CTLA-4.

Establishing the relationship between relapse-free survival and overall survival in adjuvant high-risk radically resected cutaneous melanoma

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We sought to assess the relationship between relapse-free survival (RFS) and overall survival (OS) in high-risk cutaneous melanoma patients, given recent adjuvant trials reporting mature RFS but immature OS.

A systematic literature review of bibliographic databases, clinical trial registries, and conference reports was performed. Endpoints of interest were RFS and OS. The relationship between the dependent variable (OS) and the independent variable (RFS) was assessed using weighed linear regression (WLR) of the log of the hazard ratios (LHR). As a sensitivity analysis, the COMBI-AD trial was removed to determine the influence of dabrafenib+trametinib (D+T). Model robustness was validated using a leave-one-out analysis. This was repeated for each trial, and the OS predicted by the leave-one-out model was compared with that of the left-out trial.

Both RFS and OS were reported in 18 studies assessing D+T, ipilimumab, vemurafenib, chemotherapy, and interferons. The WLR model was $LHR_{OS} = 0.03+0.89\ LHR_{RFS}$, meaning that on average a 1 unit increase in LHR_{RFS} corresponds to a 0.89 unit increase in LHR_{OS} (i.e. the risk reduction with the included melanoma treatments was approximately the same for OS and RFS). The correlation coefficient (CC) was 0.74, indicating a strong positive linear relationship between LHR_{OS} and LHR_{RFS}. Removing COMBI-AD led to a similar relationship, with a slope of 1.06 and a CC of 0.64. In the leave-one-out analysis, the predicted OS was within the observed 95% confidence intervals for all trials.

The WLR showed a strong positive linear relationship between LHR_{OS} and LHR_{RFS} with a nearly 1:1 correspondence. These results are consistent with those published by Suciu et al, 2018 (JNCI). This model may be used for the analysis of the new wave of adjuvant melanoma trials, which have reported RFS but still immature OS.

Secreted YB-1 - a novel tumour marker and functional player in melanoma progression

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Secreted factors play an important role in intercellular communication and are therefore not only indispensable for the regulation of various physiological processes but can also decisively advance the development and progression of tumours. In the context of inflammatory disease, the oncogenic transcription and translation factor Y-box binding protein 1 (YB-1) can be actively secreted promoting cell proliferation and migration. Based on an increased YB-1 expression during melanoma progression, secretion of YB-1 by melanoma cells and its functional effects as well as its potential usefulness as a melanoma marker were to be analysed in this study. Intriguingly, we can show that in contrast to benign cells of the skin such as melanocytes, fibroblasts or keratinocytes, melanoma cells can actively secrete YB-1. YB-1 secretion seems to correlate with the stage of melanoma progression and depends on a calcium- and ATP-dependent non-classical secretory pathway leading to the occurrence of YB-1 in the extracellular space as a free protein. Along with an elevated YB-1 secretion of melanoma cells in the metastatic growth phase, extracellular YB-1 exerts a stimulating effect on melanoma cell migration, invasion as well as tumourigenicity.

These data suggest that extracellular YB-1 secreted by melanoma cells plays a functional role in melanoma cell biology stimulating metastasis and may serve as a novel tumour marker in malignant melanoma.

Development of a Humanized Mouse Model of Melanoma

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Syngeneic mouse models of cancer have proven to be invaluable in cancer research, but limitations are present due to their inability to study human tumors. Patient derived xenografts have utilized human tumors implanted into immunodeficient mice. This lack of an immune system enables engraftment, but creates a model with limited translational value. Utilizing the MISTRG6 mouse system, with knocked in human genes for M-CSF, IL-3, SIRPα, thrombopoietin, GMCSF, and IL6, we have created a humanized mouse model of melanoma (mel).

We collect tumor and bone marrow (BM) from consenting patients undergoing resection of stage IIIC+ mel. The BM CD34+ cells are isolated. CD34+ cells are injected into the liver. At 8 weeks flow cytometry is done to characterize the percent CD45^{+}human hematopoietic cells (hCD45+) out of total CD45+ cells. Mice with ≥5% hCD45+ cells are considered humanized and engrafted with matched patient mel.

We have humanized mice from 3 patients. Mel growth in humanized MISTRG6 tumors was accelerated as compared to matched tumors grown in non-humanized mice. We see hCD45 IHC staining in human tumors and humanized MISTRG6 tumors, with no staining in immune deficient mice tumors. For 2 of 3 patients, whole exome and RNA sequencing has been done for all samples (human mel, non-humanized/ humanized MISTRG6 mel). To date, neoantigen prediction was done on all samples from 1 patient, and demonstrated 70 shared neoantigens with predicted MHCI binding affinity <500 nM. In addition, single cell RNA sequencing library creation/analysis is underway in order to compare the characteristics of the CD45 infiltrate and tumor subpopulations.

A humanized MISTRG6 mouse model allows for evaluation of cancer immunotherapeutics for human mel, and specifically therapy relying on targeting of neoantigens. This model has the potential to lead to true personalized medicine in the era of immune modulating therapies for mel.

Telomere length and survival in primary cutaneous melanoma patients

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Telomere repeats at chromosomal ends, critical to genomic integrity, undergo age-dependent attrition. Telomere length, a polygenic trait, has been associated with risk of several disorders including cancers. In contrast to association of long telomeres with increased risk of several cancers, including melanoma, emerging reports suggest that short telomeres predict poor survival in patients with different cancers. In this study based on 1019 stage I and II cutaneous melanoma patients, we show an association between the patients with short telomeres and poor melanoma-specific survival (HR 2.05, 95%CI 1.33-3.16) compared to patients with long telomeres. Due to inverse correlation between age and telomere length (r -0.19, P<0.0001), we stratified the patients into quantiles based on age at diagnosis and also carried out age-matched analysis. The effect of short telomeres on survival was carried out using multivariate Cox regression that included composite genetic risk score computed from genotyping of the patients for telomere-length associated polymorphisms. The effect of decreased telomere length on poor melanoma-specific survival was particularly strong in patients within the age quantile below 30 years (HR 3.82, 95%CI 1.10-13.30) and between 30-40 years (HR 2.69, 95%CI 1.03-7.03). Our study shows that in contrast to increased melanoma risk associated with increased telomere length, decreased telomere length predicts poor survival in melanoma subgroups.

EGFR/UPAR DANGEROUS LIAISON AS DRUGGABLE TARGET TO OVERCOME VEMURAFENIB ACQUIRED RESISTANCE IN MELANOMA CELLS.

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BRAF inhibitor (BRAF-I) therapy for melanoma patients harboring the V600E mutation is initially highly effective, but almost all patients relapse within a few months. Understanding the molecular mechanisms behind BRAF-I responsiveness and acquired resistance is therefore an important issue. Here we identified an unpredicted mechanism of reduced sensitiveness to the BRAF-I Vemurafenib,

driven by elevated levels of urokinase type plasminogen activator receptor (uPAR). Previously, we reported that human melanoma cells over-express uPAR, which co-localizes with EGFR thereby inducing a glycolytic phenotype in normoxic conditions.

In this study, we demonstrated that cells with different uPAR expression levels show variable sensitivity to Vemurafenib. In addition, we cultured BRAF-mutant melanoma cells in the presence of Vemurafenib until the emergence of resistant derivative and describe a promising combinatorial strategy to address acquired resistance to monotherapy by targeting uPAR and EGFR interaction with an integrin antagonist peptide.

Lastly, we retrospectively assessed the impact of EGFR or uPAR expression levels on clinical outcomes in 6 patients with metastatic melanoma treated with vemurafenib. We found significant detectable uPAR and EGFR levels in 4 relapsed patients and two of them exhibited very high levels of mRNA relative to both markers.

We demonstrated that resistance to Vemurafenib depends on uPAR-EGFR interaction, whose inhibition may convert the transient response to BRAF-I to a stable response and be a novel combinatorial strategy to overcome therapeutic resistance in melanoma patients.

Our data suggest that uPAR may be a useful biomarker to identify patients with BRAF-mutant melanoma who will or will not respond to BRAF-I.

Implications of a 31-gene expression profile (31-GEP) test for cutaneous melanoma (CM) on AJCC-based risk assessment and adjuvant therapy trial design

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As options in the adjuvant setting expand for CM patients, accurate assessment of risk of relapse is increasingly important. A 31-GEP prognostic test determines 5-year risk of recurrence, categorizing patients as low (Class 1; 1A lowest) or high risk (Class 2; 2B highest). This study evaluated risk stratification by the 31-GEP following AJCC staging, and the potential application of a Class 2 result as enrollment criteria in adjuvant therapy trial design. Archival primary CM tumor samples with clinical data from 18 centers were collected, restaged to AJCC 8th edition (n=173 Stage II-IIIA), and assessed using the 31-GEP test. Class 1A and 2B-predicted melanoma-specific survival (MSS) rates were compared to those from AJCC (8th ed). In the GEP cohort, patients eligible for therapy but at low risk (Stage IIIA) or those under consideration for adjuvant therapy trials (Stage II) had 5-year MSS rates of 90.7% and 90.9%, respectively, consistent with reported AJCC MSS rates. Stage II-IIIA Class 1A cases had a MSS rate of 100% (equivalent to AJCC Stage IA MSS). Stage II-IIIA Class 2B cases had a MSS rate of 84.7%, similar to AJCC Stage IIIB risk. To determine if a Class 2 result could optimize clinical trial accrual, relapse rates from an extended cohort and 31-GEP results were used for two-arm trial sample size calculations. To provide 90% power to detect a HR of 0.6 with 3 years of follow-up (alpha=0.05) in line with recent trials, 683 Stage II-IIIA patients are required for randomization. However, sample size could be reduced by 26% to 504 patients by focusing enrollment on patients with high risk (Class 2) of recurrence. These data suggest the 31-GEP can identify Stage II-IIIA patients who are appropriate candidates for adjuvant therapy, including during future trial design with earlier stage patients.

NK cells in the context of MHC class I downregulation in stage IV melanoma patients treated with anti-PD-1

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A known mechanism to anti-PD-1 therapy is the downregulation of major histocompatibility complex (MHC) class I expression, which prevents T cell recognition of the tumor. This study determined the relationship between natural killer (NK) cells and clinical response to anti-PD-1 therapy in metastatic melanoma. Twenty-five anti-PD-1 treated metastatic melanoma patients were categorized into responders (complete response (CR)/partial response (PR)/stable disease (SD)≥6 mo, n=13) and non-responders (SD<6 mo/progressive disease (PD), n=12) based on RECIST response. Whole transcriptome sequencing, multiplex immunofluorescent staining and spatial distribution analysis were performed on pre-treatment and on a subset of early during treatment tumor samples. Cytotoxic assay was performed using NK cells treated with anti-PD-1 or with isotype control and co-cultured with 3 different melanoma cell lines and with K562 cells (leukemia cell line). Differential expression analysis identified 9 upregulated NK cell specific genes (adjusted p<0.05) in responders (n=11) versus non-responders (n=10). Immunofluorescent staining of biopsies confirmed asignificantly higher density of intra- and peri-tumoral CD16+ and granzyme B+ NK cells in responding patients (*p*< 0.05). Interestingly, NK cells were in closer proximity to tumor cells in responding PD-1 treated patients compared to non-responding patients. Patients who responded to anti-PD-1 therapy, despite MHC class I loss had higher NK cell densities than patients with low MHC class I expression. Lastly, functional assays demonstrated PD-1 blockade induces an increase in NK cells' cytotoxicity. Here we show that NK cells may play an important role in mediating response to anti-PD-1 therapy, including in a subset of tumors downregulating MHC class I expression.

Tumor *CDKN2A*-associated *JAK2* loss predisposes to immunotherapy resistance

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In the past years, immune-modulating antibodies targeting the inhibitory T-cell receptor PD-1 (immune checkpoint therapy, ICBT) have revolutionized cancer treatment, demonstrating clinical benefits in several malignancies, including melanoma. However, more than half of the patients do not respond and therapy resistance mechanisms are still poorly understood. Recently it was shown that the genetic inactivation of the JAK1/2-STAT1 signaling pathway, protecting tumor cells from the anti-proliferative and pro-apoptotic activity of T cell-derived IFNγ, plays an important role in the development of resistance to ICBT. Generally, JAK2-deficient melanoma cells show an inactivating mutation in one *JAK2* allele and loss of the second allele. In this work, we aimed to define the genetic evolution of JAK2 deficiency in melanoma cells.

SNP array analyses of 46 melanoma cell lines revealed the presence of large deletions on one allele of chromosome 9p encompassing the *JAK2* gene. In more than 75% of the cases loss of *JAK2* was associated with a deletion of *CDKN2A*, encoding for tumor suppressor p16. Loss of *CDKN2A* is a frequent well-known early event in melanoma development and other cancer entities. When we assessed The Cancer Genome Atlas (TCGA) for the melanoma tissue data set, we detected a significantly increased proportion of samples carrying losses of both *JAK2* and *CDKN2A*, compared with samples with just one or no deletion of these genes. The same co-deletion of *JAK2* and *CDKN2A* was detected also in a large fraction of samples from different tumor types, including lung squamous cell carcinoma and bladder urothelial carcinoma, which are also approved for ICBT. Our result shows that tumors harboring allelic *CDKN2A* deletions may be more prone to develop resistance to immunotherapy due to associated *JAK2* allele losses.

Role of melanoma MHC fucosylation in CD4+ T cell-mediated tumor suppression

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Despite the striking efficacy of immunotherapies in some melanoma patients, poor responsiveness in many patients is still a challenge. Thus, further studies are crucial for improving the efficacy of immunotherapies. We previously discovered that increasing fucosylation in melanoma reduces tumor growth, metastasis, and increases tumor-infiltrating lymphocytes (TILs) (Lau et al., Sci Signal 2015). Thus, augmenting fucosylation in melanoma tumors might enhance anti-tumor immunity and the efficacy of immunotherapies. In this study, we aim to determine the i) TILs affected by tumor fucosylation, and ii), underlying molecular mechanism. To identify TILs affected by fucosylation, we profiled TILs from tumors of control- or L-fucose-fed syngeneic mice. L-fucose suppressed tumor growth by ~60% and increased total TILs by 10-50-fold vs. controls. Of the TILs, CD4+/CD8+ T cells doubled in response to L-fucose. Depletion of CD4+, but not CD8+, T cells restored tumor growth, suggesting that CD4+ T cells are required for L-fucose-triggered tumor suppression. CD4+ T cell depletion abrogated L-fucose-triggered infiltration of NK, dendritic, and CD8+ T cells, suggesting that these cells are mediators of L-fucose-triggered tumor suppression. To determine underlying mechanism(s), we used mass spectrometry to identify fucosylated proteins in melanoma that might contribute to fucosylation-triggered immune responses. We identified 2 MHC proteins, HLA-A and HLA-DRB1. Knockdown studies revealed HLA-DRB1, but not HLA-A, as required for L-fucose-triggered suppression in vivo. Initial data suggest that fucosylation affects cell surface presentation of HLA-DRB1. Further roles HLA-DRB1 fucosylation will be discussed. Our data indicate that fucosylation of HLA-DRB1 triggers CD4+ T cell-dependent TIL recruitment and tumor suppression, highlighting a potential approach for improving immunotherapies in melanoma.

Patient profiles and treatment patterns among advanced melanoma patients treated in the US community oncology setting: A retrospective, observational study

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Clinical trials have affirmed the medical benefit of immunotherapies and targeted therapies for advanced melanoma. Limited research has explored how these trends are manifesting in a real-world setting.

This was a retrospective observational study of adult advanced (unresectable or metastatic) melanoma pts treated within a network of community oncology practices in the United States between 1/1/2014 – 12/31/2016, with follow-up until 12/31/2017. Pts with other primary cancers, clinical trial participants and those without 2 follow-up visits in a clinic utilizing the full electronic healthcare record (EHR) capacities were excluded. Study data were sourced from structured and unstructured fields of the EHR. Descriptive analyses were performed to assess patient and treatment characteristics in the first-(1L), second-(2L) and third-line and beyond (3L+) settings. 484 pts were eligible (47.9% aged ≤ 65 years; 66.7% male; 93.8% Caucasian; 32.0% with brain metastases). In 1L, 37.0%, 26.4%, 19.8% and 4.1% of pts received anti-PD1 monotherapies (anti-PD1), ipilimumab (ipi), BRAF/MEK combination therapy (BRAF/MEK combo) or ipilimumab/nivolumab, respectively. 1L BRAF/MEK combo pts were younger than anti-PD1/ipi pts (median age 61 vs.

70/67 years, respectively; P < 0.0001). 259 pts advanced to 2L: 82.0% of 1L ipi pts vs. 28.5% and 57.3% of 1L anti-PD1 and BRAF/MEK combo pts. In 2L, 54.1% of pts received anti-PD1, 11.2% BRAF/MEK combo and 10.8% ipi. Among 2L pts, 86 advanced to 3L+: 34.0% received anti-PD1, 16.0% BRAF/MEK combo and none received ipi.

Between 2014-2017, immunotherapies and BRAF/MEK combo were the predominant treatments. Future research can explore how underlying characteristics of pts and increased adoption of anti-PD1 combination therapy influence treatment choice, as well as clinical and pt-reported outcomes.

Pembrolizumab in Real-World Oncology Clinical Practices: Patient Characteristics and Outcomes in Advanced Melanoma

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In patients (pts) with advanced melanoma (AM), pembrolizumab (PEM) has shown to improve long-term survival. This study aimed to examine pt characteristics and outcomes for those treated with PEM in the US real-world (RW) oncology clinical practices. Adult pts with AM who initiated PEM between 1/1/2014 and 12/31/2016 were identified retrospectively from the electronic healthcare record data of US Oncology Network and followed through 12/31/2017. Pts in clinical trials were excluded. Pt demographic, disease, treatment characteristics and reasons for PEM treatment discontinuation were described. Overall survival (OS) and physician-reported progression free survival (PFS) from PEM initiation were analyzed using Kaplan Meier methods. Three hundred and three pts were included this analysis, with a median follow-up of 13.8 months (mos, range= 0.1-38.4). Of the 303 pts, 119 initiated PEM in first-line, 131 in second-line, and 53 in third-line or beyond. Median age at PEM initiation was 67 years; 29% had elevated lactate dehydrogenase (LDH); 25% had brain metastases and 69% had an ECOG performance status of 0-1, 13.5% ECOG 2+ and 17.5% missing. The most common reason for PEM discontinuation was disease progression (36%), with 13% due to treatment-related toxicity or death respectively and other 38%. Median PFS from PEM initiation was 4.9 mos (95% CI=3.9-6.9) with 12 and 24-month PFS of 39.1% (95% CI=33.4-44.7%) and 29.2% (95% CI=23.2-35.4%). Median OS was 29.3 mos (19.6-not reached) with 12- and 24-month OS of 64.2% (95% CI= 58.2-69.5) and 53.0% (95% CI=46.2-59.3). In multivariable analyses, PEM at an earlier line of therapy, no brain metastases, and normal LDH predicted better PFS and OS. The study provides insights into pts with AM treated with PEM in the RW. The findings support the effectiveness of PEM for the treatment of a heterogeneous AM pt population.

Immunotherapy and Targeted Therapy in BRAF Mutant Melanoma: Comparative Patient Characteristics and Outcomes in Real World US Oncology Clinical Practices

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Pembrolizumab (PEM) and the combination of BRAF/MEK inhibitors (BM) have significantly improved patient (pt) OS in advanced melanoma (AM). However, limited data exist whether immunotherapy or targeted agents should be the first-line (1L) choice for BRAF(+)AM. We aimed to compare pt characteristics and outcomes in BRAF(+)AM pts treated with 1L PEM vs. 1L BM using real world (RW) data. Adult pts with BRAF(+)AM who received ≥1 dose of PEM or BM in 1L between 1/1/2014 and 12/31/2016 were identified from combined EHR data of McKesson and Flatiron, and followed up to 10/31/2017. Pts in clinical trials were excluded. Pt characteristics were compared between cohorts in univariate analysis. OS, rwPFS, and time to treatment failure (TTTF) from treatment initiation were compared using Kaplan Meier (KM) analysis. Multivariate Cox model was used to analyze the OS. 310 pts were included, 86 with PEM and 224 with BM. No significant differences exist in race, gender, BMI, stage, Charlson comorbidity index, and brain metastasis between the two cohorts. However, the PEM cohort were older, had better ECOG performance status, and were less likely to have elevated LDH vs the BM cohort. Using KM method, rwPFS and TTTF were similar between the two cohorts; OS favored PEM with a median of 22.6 months (95% CI, 19.6-not reached) vs. 13.5 months (95% CI, 11-16), 1-year OS rate of 75% (95% CI, 64%-84%) vs. 53% (95% CI, 46%-60%) compared to BM, and logrank test p=0.004. Adjusting for brain metastasis, ECOG, age, and BMI using multivariate Cox model, PEM was favored over BM in OS (HR=0.5, p=0.03). In this study, a small set of pt characteristics differ significantly between PEM and BM cohorts. Although rwPFS and TTTF were similar, OS was significantly improved in pts receiving 1L PEM vs. 1L BM. Future studies are needed to confirm the results.

Follow-up analysis of MASTERKEY-265 phase 1b (ph1b) study of talimogene laherparepvec (T-VEC) in combination (combo) with pembrolizumab (pembro) in patients (pts) with unresectable stage IIIB–IVM1c melanoma (MEL)

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Previous findings from the MASTERKEY-265 ph1b study showed that the combo of T-VEC and pembro was well tolerated and resulted in a high overall response rate (ORR) of 62% with a complete response (CR) rate of 33% in pts with advanced MEL. The 18-month (mo) progression-free survival (PFS) and overall survival (OS) rates were 66% and 86%, respectively (Ribas et al. *Cell*.2017;170:1109–19). Here, we report the results of the follow-up efficacy analyses.

MASTERKEY-265 ph1b trial was an open-label, single-arm study that enrolled pts who had unresectable, stage IIIB–IVM1c MEL with injectable, measurable lesions and no prior systemic treatment. T-VEC was administered intralesionally at the approved dosing starting day 1 of wk 1. Pembro (200 mg) was administered intravenously Q2W beginning on day 1 of wk 6. The maximum treatment period was 2 years. The primary endpoint was dose-limiting toxicities; key secondary endpoints included ORR and PFS per modified irRC, OS, and safety.

As of the data cutoff (Jun 11, 2018), all 21 pts enrolled were off treatment; 6 died and 15 are in long-term follow-up. The median follow-up time was 36.8 (1.4 – 39.6) mos. 2 pts' tumor response changed from previous partial response/stable disease to CR. The ORR improved to 67% (14/21 pts, 95% Cl, 43.0%–85.4%) with a CR rate of 43% (9/21 pts). Among the 14 responders, 12 (57%) remain in response, including the 9 with CR. Median PFS and OS were not reached at the data cutoff. 36-mo PFS and OS rates were 53.6% and 71%, respectively. No additional safety signals were detected.

The combo of T-VEC and pembro is well tolerated and induces durable responses in the majority of pts in the ph1b study. The corresponding randomized ph3 study has completed enrollment and is currently ongoing.

The changing role of SLAMF6 immune receptor splice isoforms – a new regulatory mechanism

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The SLAM family of receptors are homotypic binders highly conserved in hematopoietic cells. SLAMF6 is abundantly expressed in CD8 T cells and thus of interest for its role in tumor immune response. The human SLAMF6 gene has four splicing isoforms of which the 'canonical' long sequence, and the short SLAMF6Δ^{17-65} missing part of exon 2- containing part of the receptor's IgV domain, are of interest. **In this work, we set to evaluate the immunological modulatory effect of the long and the short isoforms of SLAMF6.**

We found that all SLAMF6 isoforms (identified by RT-PCR) are constitutively present on T cells, regardless of activation/differentiation state. However, a difference in the level of isoform transcripts was found in CD8 subsets in healthy donors. Specifically, SLAMF6 long isoform has relatively low expression in naïve and central-memory cells, but much higher on effector and effector-memory cells.
To characterize their net-effect, we aberrantly expressed each isoform in melanoma followed by co-culture with cognate T cells. Melanoma cells expressing canonical SALMF6 inhibited cognate TIL function, while an enhanced response was observed with short isoform- expressing melanoma.

To generate Jurkat cells expressing only the short SLAMF6 isoform we used CRISPR-Cas9, knocking-out the long isoform. The sole expression of SLAMF6Δ^{17-65} was associated with a significant increase in cytokine secretion.

Overall, we found: (1) dynamic expression of SLAMF6 isoform transcripts in CD8 T cells and (2); that the short and long isoforms have a strong but opposing impact on T cell function. **We suggest that manipulation of SLAMF6 splicing can be exploited for cancer immunotherapy**. And indeed in preliminary experiments, using antisense-oligonucleotides designed to block spliceosome binding, a shift in SLAMF6 splicing pattern was achieved.

Adjuvant Therapy With Pembrolizumab (Pembro) vs Placebo in Resected High-Risk Stage II Melanoma: The Phase 3 KEYNOTE-716 Study

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In KEYNOTE-054 (*NEJM* 2018;378:1789-801), adjuvant pembro showed significantly longer recurrence-free survival vs placebo in resected stage III melanoma. KEYNOTE-716 is a 2-part randomized, placebo-controlled, multicenter study of adjuvant pembro in surgically resected high-risk stage II melanoma (NCT03553836). Eligible patients are ≥12 years of age with newly diagnosed, completely resected stage IIB/IIC cutaneous melanoma, as defined by AJCC 8th edition (including negative sentinel lymph node biopsy and no evidence of distant metastasis). Patients cannot have uveal or mucosal melanoma or have received prior treatment, including radiation, for melanoma beyond resection of primary melanoma within 12 weeks of the start of study therapy. In part 1 (double-blind), patients will be randomized 1:1 to receive pembro 200 mg (age ≥18 years); 2 mg/kg (age 12-17 years; maximum dose, 200 mg) or placebo Q3W for 17 cycles. Stratification: 1 stratum for pediatric patients (12-17 years); 3 strata for adult patients per T stage (T3b/T4a/T4b). Study treatment will begin within 12 weeks of complete resection. Tumor imaging will be performed Q24W on treatment, at the end of treatment, every 6 months for the first 3 years off treatment, and then yearly for up to 2 years or until recurrence (up to 5 years total imaging). AEs will be graded per NCI CTCAE v4.0. In part 2 (unblinded), patients with confirmed recurrence may be rechallenged (received pembro in part 1) or crossed over to pembro (received placebo in part 1). Resected local/distant recurrence or unresectable disease will be treated for an additional 17 or 35 cycles, respectively. Tumor imaging in part 2 will occur Q12W on treatment. Primary end point is recurrence-free survival. Secondary end points are distant metastasis-free survival, OS, and safety. Approximately 954 patients will be enrolled.

IFNγ-Activated Dermal Lymphatic Vessels Inhibit Cytotoxic CD8^{+} T cells in Melanoma

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Mechanisms of immune suppression in peripheral tissues counteract protective immunity to prevent immunopathology and are coopted by tumors for immune escape. While lymphatic vessels facilitate T cell priming through dendritic cell and antigen delivery to lymph nodes, they also exert PD-L1-dependent immune suppressive effects in lymph nodes at steady state, and facilitate melanoma metastasis. We hypothesized that peripheral lymphatic vessels activate immune suppressive mechanisms, e.g. PD-L1, to limit local effector CD8^{+} T cell accumulation in melanoma. We first demonstrate that non-hematopoietic, non-tumor PD-L1, primarily expressed by lymphatic and blood endothelial cells, limits CD8^{+} T cell accumulation in melanoma. We find that IFNγ produced by tissue infiltrating, antigen-specific CD8^{+} T cells is sufficient to activate lymphatic vessel PD-L1 expression. As such, disruption of IFNγ-dependent crosstalk through lymphatic-specific loss of IFNγR boosts T cell accumulation in melanoma leading to CD8^{+} T cell-mediated tumor control and improved overall survival. Importantly, robust lymphatic vessel-dependent tumor control was revealed in the context of murine melanomas with significant UVB-induced somatic mutational burden, consistent with our model of cytotoxic T cell-activated negative feedback. Consequently, we present IFNγ-mediated activation of tumor-associated lymphatic vessels as a new immune checkpoint in melanoma that contributes to adaptive immune resistance. Further, we suggest that understanding context-dependent lymphatic vessel function will continue to reveal novel mechanisms of immune control and metastasis in melanoma.

Metabolic regulator PGC1α suppresses WNT5A-YAP axis to regulate melanoma metastasis and resistance to BRAFV600E inhibition

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Despite the impressive initial response to BRAF[V600E] inhibitors, rapid development of resistance dwarfs the long-term benefits of these targeted therapies. Furthermore, therapy-resistant tumors evolve to be even more invasive and metastatic; however, the mechanism underlying this resistance-associated aggressiveness is largely unknown. Our lab previously identified that heterogeneous levels of the metabolic regulator PGC1α within melanoma dictate their differential metastatic propensity, with PGC1α-low population adapting an invasive phenotype causing systemic metastasis. Interestingly, we recently found that melanoma cells resistant to

BRAF[V600E] inhibitor PLX4032 significantly enhanced their migratory ability, concomitant with reduced expression of PGC1α. Down-regulation of PGC1α in the PLX4032-resistant cells induced WNT5A expression and secretion, which in turn promoted the stabilization and activation of YAP transcriptional coactivator through non-canonical WNT pathway. Depletion of YAP or antagonizing WNT5A in resistant cells with lowered PGC1α expression greatly suppressed their migration *in vitro* and metastasis *in vivo*. Furthermore, in clinical melanoma samples, YAP activity, as reflected by expression of YAP signature genes, not only inversely correlated with PGC1α levels but also predicted patient survival post PLX4032 treatment. In conclusion, we identified that decreased expression of PGC1α in treatment resistant melanoma cells contributes to their aggressive phenotype through WNT5A-mediated YAP activation, suggesting that WNT5A and/or Hippo-YAP pathway would be novel therapeutic targets for combating resistance to BRAF[V600E] inhibition.

Overcoming MAPK inhibitor resistance by targeting DNA damage repair mechanisms in malignant melanoma
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The success of BRAF and MEK inhibitor (MAPKi) treatment in melanoma therapy is limited by the development of resistance. Cancer cells frequently show altered capacity of DNA damage repair; however the influence of DNA repair machinery on melanoma therapy resistance is less analyzed so far.

We compared melanoma cells before and after acquisition of resistance to MAPKi regarding their sensitivity towards DNA damaging treatments and found an enhanced susceptibility of MAPKi resistant cells to some genotoxic drugs. The expression analysis demonstrated a suppressed level of several DNA damage repair related genes and proteins in melanoma cells resistant to targeted therapy. This alteration during MAPKi resistance development was accompanied by reduced DNA repair capacity upon genotoxic stress. We further found that the inhibition of compensatory DNA repair pathways impair the genomic stability of melanoma cells and thereby restore the sensitivity of MAPKi resistant melanoma cells to MAPKi treatments.

Our data demonstrates a therapeutic potential of combined inhibition of specific DNA repair pathways in melanoma cells in addition to MAPKi treatment for overcoming MAPKi resistance.

Characteristics of Pyrexia with Encorafenib (ENCO) Plus Binimetinib (BINI) in Patients with *BRAF*-mutant Melanoma
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Combined BRAF/MEK inhibitor therapy is a standard of care in advanced *BRAF*[V600]-mutant melanoma. Although all 3 available combinations share class-related toxicities, certain compound-specific adverse events are qualitatively distinct. Pyrexia with previously approved combination regimens is variably serious, with associated symptoms that may include chills, dehydration, hypotension, renal failure, or syncope. Here, we present the characteristics of pyrexia in the phase 3 COLUMBUS study in patients (pts) who received ENCO 450 mg QD + BINI 45 mg BID (ENCO+BINI). Pts with *BRAF*[V600] metastatic melanoma were randomized to oral ENCO+BINI, ENCO 300 mg QD, or vemurafenib 960 mg BID. Safety was analyzed in pts who received ≥1 study drug dose and ≥1 postbaseline assessment. Pyrexia was a grouped term that included individual preferred terms for increased body temperature, hyperpyrexia, hyperthermia, and pyrexia. Pyrexia occurred in 18% of pts in the ENCO+BINI arm (n=192; grade 1/2/3/4: 12%/2%/4%/0), leading to study discontinuation in 1% and dose modification in 4%. The median time to first onset of pyrexia was 85 d (range, 2–545 d); onset at <3, 3 to <6, 6 to <9, 9 to <12, and ≥12 mo occurred in 9%, 2%, 2%, 3%, and 2% of pts. Among the 8 pts with grade 3 pyrexia, 5 had multiple episodes, of which 74% were grade 1/2. No pts with grade 3 pyrexia had concurrent chills or dehydration. Treatment included antibiotics and/or antipyretics. Grade 3 pyrexia was concomitant with progressive disease (PD) in 3 pts and underlying infection in 2 pts (1 of whom also had PD). In summary, pyrexia with ENCO+BINI treatment was of low frequency,

with few grade 3 events, and characteristics that fundamentally differed from those observed with other available BRAF/MEK inhibitor combinations in temporal distribution, recurrence, and association with events such as chills and dehydration.

A lineage approach uncovers a novel regulator of metastatic melanoma proteostasis

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Metastatic melanoma is an aggressive cancer of the melanocyte with few therapeutic options, highlighting a need for new therapeutic targets. We hypothesized that melanoma cells can adopt a global, embryonic-like phenotype to facilitate progression, reasoning that cellular characteristics that enable embryonic melanoblasts (melanocyte precursors) to migrate and colonize distant sites, can be co-opted by melanoma cells to promote metastasis. Using the *i*Dct-GFP mouse, which expresses GFP in both immature melanoblasts and mature melanocytes, we isolated GFP$^+$ cells from pre- and post-natal developmental stages (E15.5, E17.5, P1, P7) and performed RNA sequencing to uncover novel melanoblast gene expression. We found re-expression of identified melanoblast genes in a sub-population of metastatic melanoma patients, and uncovered a gene signature that predicts patient survival; experimental metastases and anchorage-independent growth assays confirmed their role in metastasis. The top candidate, *KDELR3*, encodes a retrograde trafficking protein involved in the Endoplasmic Reticulum (ER) Stress Response. Treatment with chemical inducers of ER stress confirmed *KDELR3* knockdown (KD) sensitizes cells to ER stress-induced apoptosis. Quantitative mass spectrometry of cycloheximide-treated cells revealed global attenuation of protein degradation in *KDELR3* KD cells. Attenuated proteins overlapped with those identified following knockdown of gp78, an E3 ubiquitin ligase in ER-Associated Degradation (ERAD). We found KDELR3 and gp78 interact, and confirmed upregulation of KAI1, a known gp78 substrate and metastasis suppressor. In this study we demonstrate that melanoma cells co-opt a melanoblast phenotype to facilitate progression, and establish a novel role of *KDELR3* as a melanoblast gene that regulates ER-stress and ERAD, highlighting these pathways as potential therapeutic targets in metastatic melanoma.

Adaptive counter strike mechanism of the immune system

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ADAR1 is a constitutively expressed (p110) RNA editing enzyme that can be induced by alpha-interferon (p150). We previously reported that p110 is downregulated in the transition from primary to metastatic melanoma. Silencing of ADAR1 facilitates proliferation and invasion, and renders melanoma resistant to antigen-restricted T cells. Using modified Boyden chambers, here we show that silencing or overexpression of ADAR1 decreases or increases, respectively, specific T-cell migration toward melanoma cells. This is supported by robust congruent alterations in chemokine production as demonstrated by chemokine arrays. TCGA studies confirm direct prognostic implications of these chemokines on overall survival of melanoma patients. Mechanistically, ADAR1 controls major chemokine expression and chemotactic potential through regulation of microRNAs, such as hsa-miR-425. Importantly, co-cultures using HLA compatible and –incompatible T-cells and melanoma cells, or CRISPR-knockouts of beta2-microglobulin, demonstrate a recognition-restricted, IFNg-dependent, induction of p150. This is visualized in-situ using dynamic cell blocks and observed in western blots. The IFNg-induced p150, or its ectopic overexpression in melanoma cells facilitates T-cell migration in an RNA-editing dependent manner. RNAseq of 40 melanoma tumors prior to anti-PD-1 therapy shows a statistically significant correlation between the presence of p150 but not p110, and T cell inflammation signature.
Collectively, ADAR1 downregulation facilitates a pro-tumor environment by limiting the amount of T-cells that migrate to the area hence creating a cold tumor. On the other hand, antigen-specific T-cells forces an adaptive counter attack by eliciting a positive feedback loop through by inducing p150 in the surviving tumor cells, which drives the expression of several chemokines to increase the chemotactic potential and thereby the influx of new T-cells.

Utilization of Real World Data to Assess the Efficacy of Immune Checkpoint Inhibitors (ICI) in Elderly Patients with Metastatic Melanoma

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Background: The widespread introduction of ICI in pts with metastatic disease has significantly improved outcomes. However, ICI in the elderly requires further evaluation to determine whether their survival and toxicity differ from those observed in younger cohorts. The goal of this study is to utilize real world data from the Canadian Melanoma Research Network Registry.
Methods: This retrospective observational study was performed in metastatic pts entered into a common clinical registry and who received ipilimumab alone (ipi) and nivolumab (nivo) or pembrolizumab (pembro) from 2009-2018. Demographics, extent of disease, all treatments and adverse events (AEs) were compiled. Comparisons between pts in different age cohorts were made and the potential impact of known prognostic factors was investigated using Cox proportional multivariate analyses.
Results: 153 pts over 70 were treated with ICI. 11% had only pulmonary mets; 39% had liver mets; and 14% had brain mets. 21% had a BRAF mutation. 56% were treated with ipi and 44% received nivo or pembro. The mean survival for those aged 70-79 was 9.1 M (SD=10.2) and 8.5 M (SD=8.3) for those aged 80+. In a comparative cohort of pts aged 60-69, the mean survival was 9.3 M (SD=10.4). On multivariate analysis, type of ICI, baseline LDH and BRAF status did not impact overall survival. 56 >grade 2 AEs were observed in pts on ipi: 50% colitis, 39% rash, 7% hypophysitis, 4% hepatitis. In those pts receiving nivo or pembro, 31 >grade 2 AEs were reported: 42% rash, 35.5% colitis, 13% arthritis/arthralgia, 6.5% thyroid dysfunction and 3% adrenal insufficiency.
Conclusion: ICI can be effectively utilized in pts over 70. Survival appears to be comparable to that achieved in younger cohorts; however longer follow-up is required. AE rates are similar; however rates appear higher with ipi.

AMBRA1 and Loricrin; a paradigm shift in early melanoma prognostication

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Despite recent revision of AJCC staging criteria, the identification and validation of credible prognostic biomarkers remains critical to identifying patients with high risk early stage melanomas, their subsequent counselling, stratification and follow up, including guidance on appropriate need for sentinel lymph node biopsy (SLNB).

In the present study we demonstrate the combined loss of 2 protein markers, AMBRA1 (a pro-autophagy regulatory protein) and Loricrin (a marker of terminal keratinocyte differentiation), in the epidermis overlying primary AJCC stage I melanomas identifies high-risk tumour subsets, while retained expression correlates with genuinely low-risk tumours. Specifically, semi quantitative automated immunohistochemical analysis of combined epidermal AMBRA1 and Loricrin (AMLo) expression in 2 retrospective cohorts of >400 AJCC I melanomas with a minimum 14 year follow up revealed 97.9% disease free survival (DFS) in the AMLo low-risk group versus 86.1% in the high-risk group. Sub-cohort, multivariate analysis also revealed an AMLo hazard ratio of 3.15 (95% CI 1.45-6.87, P = 0.0038) as a stronger predictor of DFS than Breslow depth (multivariate analysis 2.96 (95% CI 0.93-9.47, P = 0.0673) in AJCC stage IB patients. Moreover assessment of outcome in patients eligible for SLNB revealed a post-test probability of metastasis of 18% in the AMLo high-risk group, while only 1.4% in the low-risk group suggesting epidermal AMLo as a valuable pre-SLNB test.

Collectively these data highlight epidermal AMLo as a novel prognostic marker for AJCC stage I melanoma; a simple IHC-based marker that can integrate seamlessly into standard clinical pathways of melanoma diagnostics, informing stratified follow-up (including appropriate SLNB use), reducing patient psychological burden, promoting better healthcare resource utilization and representing a major paradigm shift in melanoma management.

Melanoma TGFβ-2 secretion leads to loss of endothelial integrity and tumour metastasis

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The identification, effective stratification and personalised treatment of patients with high risk early AJCC stage melanomas is undoubtedly hampered by a lack of credible predictive/companion biomarkers and consistently beneficial precision-based therapies. We recently identified the loss of epidermal AMBRA1 and Loricrin (AMLo) overlying AJCC stage I melanomas as a prognostic biomarker. In the present study we show loss of AMLo is also a surrogate marker for metastasis since epidermal loss also correlated with loss of AMBRA1 expression in endothelial cells lining blood vessels underlying 60 primary high risk AJCC stage I melanomas.

Bioinformatics analysis of the AMBRA1 promotor revealed the presence of TGFβ responsive elements while semi quantitative immunohistochemical analysis demonstrated significantly increased tumoural TGFβ2 expression (and not other TGFβ isoforms) was associated with AMLo high risk AJCC stage I melanomas and metastatic development, suggesting melanoma secretion of TGFβ2 leads to alterations in barrier function and metastatic spread. To test the hypothesis that targeting TGFβ2 signalling may represent a novel strategy to prevent melanoma metastasis, HUVEC endothelial cells or primary dermal lymphatic endothelial cells were treated with recombinant TGFβ2 or TGFβ2 rich supernatants derived from metastatic melanoma cell lines prior to western blot analysis for AMBRA1 and junctional protein, VE Cadherin or Claudin 5 expression. Results demonstrated significant TGFβ2-induced down regulation of AMBRA1, VE Cadherin and Claudin-5, associated with the activation of non-canonical TGFβ2 signalling. Targeting TGFβ2 /TGFβ2 receptor signalling in high risk AJCC stage I melanomas at the time of primary excision may therefore provide a novel adjuvant therapy to prevent metastasis, for which the loss of epidermal AMLo is a companion biomarker.

The gut microbiome of melanoma patients is distinct from that of healthy individuals and is impacted by probiotic and antibiotic use

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The gut microbiome has been implicated in differential responses to immune checkpoint blockade in melanoma and other cancers. However, little is known about microbiome composition of melanoma patients compared to healthy individuals or the impact of host factors in this population.

To begin to address this, we determined the composition of the fecal microbiome by 16S sequencing in 309 melanoma patients (median age 62; 59% male; 86% Stage III/IV) undergoing treatment at our center. Comparison to a cohort of healthy individuals (American Gut cohort, n=116) by ordination of unweighted UniFrac distances indicated distinct community clusters differentiating melanoma patients from healthy individuals (Anosim r=0.22; p<0.01). Higher alpha diversity was observed in melanoma patients compared to healthy individuals (p<0.01), with a higher range of diversity seen in the healthy cohort. There were no significant associations observed between microbiome composition and age, sex or body mass index among the melanoma patients.

A subset (n=113) of the melanoma patients also underwent prospective lifestyle assessments. "Biotic" use was quite common (29% antibiotics, 42% probiotics). Importantly, self-reported use of either "biotic" was associated with lower diversity of the gut microbiome (p=0.01), with significant associations observed for both antibiotics alone (p=0.05) and for probiotics alone (p=0.02). Prospective longitudinal studies are underway to assess the relationship between "biotic" use and fecal microbiome composition and diversity among patients, as well as functional studies in preclinical models.

These data provide preliminary evidence that the gut microbiome of melanoma patients is distinct from healthy individuals and influenced by use of antibiotics and probiotics, a finding with potential therapeutic implications.

Confirmation of Novel Melanoma Risk Variants on Chromosome Arm 10q

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Using a genome-wide association (GWA) study, comparing high-risk familial melanoma cases to genotypically matched controls, Teerlink et al. found that three single nucleotide polymorphisms (SNPs) in close proximity in the 10q25.1 region (rs17119434, rs17119461 and rs17119490) were associated with melanoma predisposition (Human Genetics, 2012). Likely due to their low minor allele frequencies (MAFs), traditional GWA studies have not reported associations between these SNPs and melanoma risk. We sought to confirm the relationships identified utilizing the Genes Environment and Melanoma (GEM) Study, which uses a case-control design where controls are participants with a single primary melanoma (SPM) and cases are those with multiple primary melanoma (MPM). This design is efficient at identifying rare genetic risk variants for melanoma compared to conventional population-based case control methods. In GEM, no genotyped participants were homozygous for the minor allele for these three SNPs, so all odds ratios (ORs) represent heterozygous versus homozygous major allele genotypes. The SNPs were in high linkage disequilibrium with each other (≥0.92) and had MAFs between 0.012-0.013 for cases and between 0.008-0.009 for controls. Logistic regression models adjusted for

study features estimated ORs for developing MPM relative to SPM for each SNP: rs17119434 (1.59; 95% CI: 0.94-2.67), rs17119461 (OR 1.77; 95% CI: 1.06-2.97) and rs17119490 (1.70; 95% CI: 1.00-2.88). Stepwise logistic regression identified rs17119461 as producing the strongest independent association. Further analyses revealed no significant relationships between rs17119461 and host or tumor characteristics. To our knowledge, our study is the first to validate an association of SNPs in the 10q25.1 locus with melanoma risk. This relationship deserves further investigation.

DNA methylation based immune clusters are strongly prognostic in metastatic melanoma

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Immune enrichment in tumors has attracted significant attention recently for its ability to predict response to immunotherapies. However, studies concerning specific roles of different immune cell subsets present in the tumor microenvironment of metastatic melanomas are currently limited.
We sought to address this problem by using promoter CpG methylation of immune cell type specific genes (Angelova *et al.* 2015), as a tool for identifying presence of the corresponding immune cells in tumors. 180 metastatic melanoma tumors profiled using Illumina EPIC methylation array, were clustered in line of the aforementioned CpGs and 3 immune-methylation clusters displaying strong relationship with disease specific survival (DSS) in both univariate log-rank test (p = 0.002) and multivariate cox regression analysis (Cluster 1: Reference, Cluster 2: HR=1.85(1.09, 3.14); p=0.02, Cluster 3: HR=1.68(1.03, 2.74); p=0.04) were discovered. Identified immune-methylation clusters presented a complex and varying landscape of enrichment, which was further corroborated by immuno-histochemical (IHC) staining for $CD3^+$, $CD8^+$ and $CD20^+$ lymphocytes and matching gene expressions.
To validate these findings, we classified TCGA metastatic melanoma tumors into the immune-methylation clusters and observed similar gene expression and survival characteristics. Furthermore, to understand the broader implications of identified immune-methylation clusters in pan-cancer context, we classified additional 15 different TCGA cancer cohorts. Among these cohorts, similar survival differences were found in Esophageal Adenocarcinoma and Head and Neck Squamous Cell Carcinoma cohorts.
Our findings highlight the scope of cell type specific methylation to understand immune enrichment in tumors, which might be beneficial for future immunotherapeutic applications.

Hyperactive Rac1 in melanoma drives assembly of a mechanosensitive dendritic actin network to overcome growth suppression

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As a cancer notorious for metastasis, immune evasion, and drug resistance, melanoma is highly adapted to overcome growth suppression. Within melanoma's arsenal of adaptive mechanisms, the RhoGTPase, Rac1 is frequently hyperactivated and drives resistance to BRAF inhibition, PD-L1 expression for immune evasion, and metastasis in NRAS mutant tumors. To better understand Rac1's adaptive signaling in melanoma, we describe how the melanoma-enriched hyperactivating mutation, $Rac1^{P29S}$, drives proliferation in growth limiting conditions by hijacking Rac1's function as a regulator of actin polymer assembly. This proliferative advantage facilitates metastatic colonization of melanoma cells and confers insensitivity to drugs targeting the MAPK pathway. $Rac1^{P29S}$-driven proliferation depends on cell-matrix attachment, but surprisingly does not depend on integrin-mediated focal adhesion assembly and FAK, Rho, and myosin signaling. We find that $Rac1^{P29S}$ cells have massive upregulation of dendritic actin polymerization in lamellipodia and have increased cell traction forces demonstrating enhanced mechanical engagement of the cell substrate, even without the involvement of canonical adhesion formation or signaling. Proliferation driven by $Rac1^{P29S}$ depends on this dendritic actin polymerization and is sensitive to manipulation of substrate stiffness. We find that enhanced dendritic actin polymerization in $Rac1^{P29S}$ cells drives proliferation by inhibiting tumor suppressor activity of NF2/Merlin, localizing NF2/Merlin to the lamellipodia where it is highly phosphorylated and inactivated. Thus, hyperactivation of Rac1 by the P29S mutation in melanoma drives mechanosensitive dendritic actin polymerization that inactivates NF2/Merlin to sustain proliferation and overcome growth suppression imposed by drug treatment or foreign microenvironments during metastasis.

Adjuvant therapy is associated with improved overall survival in advanced melanoma patients in the United States

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The objective was to evaluate the effect of adjuvant therapy on survival of advanced melanoma patients.

Population included adult patients with advanced cutaneous melanoma (unresectable, Stage III or IV) identified in the Flatiron Health Oncology database in the United States between January 1, 2011 and February 28, 2018. Patients were required to have at least 3 months medical data prior to the advanced diagnosis (index date) and at least 2 clinical encounters on or after the index date. Overall survival (OS) after the index date was a primary outcome. Progression free survival (PFS) from the first line therapy after index date was an exploratory outcome. OS and PFS were compared between patients with and without adjuvant therapy using non-parametric log-rank test and evaluated in a Cox proportional hazards.

There was a significant difference in OS (log-rank test p < 0.0001) and no significant difference in PFS (log-rank test p = 0.6092) between patients who received adjuvant therapy (n = 155) and patients without adjuvant therapy (n = 1,147). Adjuvant therapy was associated with increased overall survival probability (hazard ratio, HR: 0.530, p < 0.0001) and had no effect on progression free survival (HR: 1.079, p = 0.5959) after adjusting for demographic and clinical characteristics.

In this study of advanced melanoma patients, results demonstrated a better OS outcome in those treated with adjuvant therapy. A future study in resected melanoma patients is recommended to further establish benefit of adjuvant therapy.

Dissecting role of ultraviolet radiation exposure patterns in [V600E]BRAF driven melanoma

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Ultraviolet radiation (UVR) is the major environmental risk factor for melanoma. A large spectrum of mutation burdens is observed in patients suggesting large variability in UVR exposure. However, how specific patterns of UVR exposure confer melanoma risk through genomic alterations remains unclear and it is not feasible to explore this in patients.

We used our mouse model of melanoma driven by [V600E]BRAF and UVR to dissect the role of different patterns of exposure in melanomagenesis by exposing mice to single dose, short-term (4 weeks) and long-term (up to 26 weeks) UVR using a broad-spectrum UVR lamp (280-380 nm). Time to first lesion was used to determine melanoma free survival and the genomic changes in the tumours were determined by whole exome sequencing (WES).

When exposed to different patterns of exposures, single shot of UVR was sufficient to accelerate melanomagenesis. Intriguingly, single shot of UVR was also sufficient to imprint a mutational signature 7, which is associated with UVR exposure. Moreover, mice with short-term UVR exposure showed significantly accelerated melanomagenesis, compared to long-term exposure. WES data revealed associations of mutation load, neoantigen load and C>T load with increasing UVR exposures. Across cohorts, *Map3k1* was the most mutated gene, but *Trp53* mutations were almost exclusively associated with long-term UVR exposure. Our results suggest that short-term UVR exposure is particularly effective in accelerating melanoma in our [V600E]BRAF mouse model. Exploring and understanding how these exposure patterns drive melanomagenesis has direct translational implications and will lead to more accurate recommendations in melanoma prevention advice.

Blood Test Parameters (BTP) to predict clinical outcomes in Metastatic Melanoma (MM) patients (pts) treated with immunotherapy (iTx)

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Routine BTP have been proposed as potential biomarkers to predict clinical benefit and avoid toxicity of iTx. Here we identify BTP associate with prognosis in MM pts treated with iTx. Single-institution review of 115 consecutive pts with MM treated with any iTx in our center from 2010 to 2017. Clinicopathological features, treatment (Tx) pattern, baseline BTP, derived absolute neutrophil-to-lymphocyte ratio (NLR) and Time to Tx failure (TTF: time from the first dose to end of Tx for any reason) were analyzed. Median (Md) age was 62 years. BRAF mt was 46%. Md follow-up was 24 m. Md TTF was 3.7m (3.1-4.1). Of the total of 207 Tx received, iTx was 45% first, 46% second and 9% third o beyond. In univariate analysis, LDH >520 (p<0.001), NLR >5.6 (p<0.001), absolute monocyte >631 (p=0.03) and relative eosinophils <0.3% (p=0.09) were associated with worst TTF on iTx and those were select as

adverse factors (AF) for combined analysis. An inverse relationship between the number of AF at baseline and TTF was seen: 1 (HR 1.5, p=0.06), 2 (HR 2.5, p<0.001), 3 (HR 3.8, p<0.001) and 4 AF (HR 4.6, p<0.001). In the AF 0-1 group (n=71), Md TTF was significantly longer with anti-PD1 than anti-CTLA4 (HR 2.0, p=0.02) without difference between combo and anti-PD1 alone (HR 1.4, p=0.3). Respect non-iTx, no difference was found with tTx 6.1m (HR 0.7, p 0.11) but oTx had shorter TTF 2.6 m (HR 2.3, p=0.01). In the AF 2-4 group (n=21), no difference was found between iTx regimens: Anti-CTLA4 vs. anti-PD1 (HR 1.0, p 0.9) or combo vs anti-PD1 (HR 1.1, p=0.8) or with non-iTx. Our real-world data confirm the prognostic value of routine baseline BTP in pts treated with iTx. In pts with favorable lab tests (AF 0-1), combination iTx did not improve outcomes as compared with anti-PD1 alone. The poor risk group (AF 2-4) had dismal prognosis irrespective of Tx selected.

Vitamin D Receptor (*VDR*) expression is prognostically significant in a cohort of 703 primary melanomas with evidence for anti-proliferative and pro-immune effects

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The genomic basis of the protective effect of vitamin D-*VDR* signalling on melanoma prognosis is not fully understood. We report *VDR* expression is a significant predictor of prognosis in a cohort of 703 treatment-naïve primary melanomas, independent of AJCC stage, tumour site, mitotic rate, and TILs (HR =0.8, P=0.008). We used transcriptomic data from the 703 primary melanomas to identify genes and pathways associated with *VDR* expression, aiming to gain insight into the genomic-wide effects of *VDR*-expression. Genome-wide correlation and enrichment analysis (Reactome FIViz) revealed that *VDR* expression is significantly (FDR<5%) and positively correlated with 2025 genes enriched for immune signalling pathways (eg. NF-kB, IFN-g, TNF and IL12-mediated signalling) and inversely correlated with 1383 genes enriched for proliferation and cell cycle pathways (eg. Mitotic prophase, Wnt signalling pathway and Mitochondrial translation). Concordantly, high-*VDR* tumours also had significantly higher pathologist-reported TILs (P<0.04) and imputed immune cells scores (P<10^{-16}) compared to low-*VDR* tumours. Interestingly, there was no significant difference in *VDR* copy number or frequency of common *VDR* polymorphisms between the low and high-*VDR* tumours. We explored the causal role of *VDR*-expression (in addition to the correlative evidence above) using a tail-vein metastasis assay, wherein metastatic load and CD3+ immune infiltrate were compared between stable-transfected B16-BL6:*VDR* mouse melanoma cells and control cells (work in progress). Taken together, our study is the first to identify the whole-genome correlates of *VDR* to be associated with pro-immune and anti-proliferative pathways. Additionally, our study aims to provide causal evidence for the pro-immune and anti-proliferative effects of tumour *VDR* expression.

Histological characteristics associated to the presence of an invasive component in lentigo maligna

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An invasive component is detected in approximately 20% of previously biopsied lentigo maligna. So far, there are not predictive features that indicate the presence of that invasive component. Some histological characteristics have been described as part of the theoretical progression model that makes a lentigo maligna eventually invasive.
Our aim was to evaluate if there are pathological characteristics in the diagnostic partial biopsy of a series of lentigo maligna that predict the presence of an invasive component in the definite excision of the lesion.
A series of 96 cases of lentigo maligna/lentigo maligna melanoma with available adequate material for histological re-examination was identified from our database. All the specimens belonging to the diagnostic partial biopsy with a diagnostic of lentigo maligna were re-examined for the presence of some pathological characteristics. Two groups were defined according to the presence or not of an invasive component. The differences between the distribution of the variables between the groups were assessed using the chi square and the Fisher exact tests. The degree of the association of the significant variables was quantified by logistic regression models. A classification and regression tree analyze was performed to arrange in order of importance the significant variables.
Out of 96 studied cases, 33 (34,4%) had an invasive component. The presence of melanocytes arranged in rows, subepidermal clefts, nests and non-CSD elastosis, were all significant histopathologic signs frequently associated to an invasive component. A prediction tree was able to discriminate situations in which >60% of cases were invasive.
Our results could be useful to anticipate the presence of an invasive component in previously biopsied lentigo maligna, what eventually can be relevant for the management.

Prognostic factors for hematogenous and lymphatic dissemination in stage I/II melanoma patients. A longitudinal study on 1177 stage I/II melanoma patients

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Metastasis spreading from melanoma develops through lymphatic or hematogeneous vessels. The role of new adjuvant therapies, image techniques during follow-up as well as lymphadenectomy can benefit from identifying who is at an increased risk of developing metastases through each path.

A longitudinal study on 1177 patients with stage I/II cutaneous melanomas was designed based on the information prospectively collected in the melanoma database of the institution. Prognostic factors for locoregional (lymphatic) and distant (hematogenous) progression were evaluated by Kaplan-Meier curves, log-rank test and Cox regression models

After a median follow-up of 75 months, 3.7% of the patients developed exclusively locoregional disease, 3.7% only distant metastasis and 5.5% both of them. An age at diagnosis older than 55 years, tumors located on head/neck or acral regions, tumor thickness and vascular invasión were associated to locoregional relapse. In contrast, tumor thickness, the absence of regression, and the presence of BRAF and TERT promoter mutations were the characteristics associated to distant metastasis.

Clinical, histological and molecular characteristics determine the pattern of progression of cutaneous melanoma. Our data support the use of BRAF and TERT promoter mutation testing to identify patients at an increased risk of distant metastasis and therefore possible candidates to adjuvant therapies.

A Phase 1 study of Neoadjuvant Combination Immunotherapy with Pembrolizumab (P) and High Dose IFN-α2b (HDI) in Locally/Regionally Advanced Melanoma

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Background: Neoadjuvant P in combination with HDI may improve clinical outcomes of high-risk patients (pts) with locoregionally advanced/recurrent melanoma (mel). **Methods:** Primary endpoint: safety of combination P-HDI. Pts were treated with P 200 mg IV every 3 weeks (wks) x 2 doses followed by definitive surgery, then every 3 wks x1 year. HDI per standard FDA approved regimen was given concurrently. Tumor and blood samples were obtained at baseline and at definitive surgery (wk 6-8), blood also at 6 wks, 3,6,12 months (mos). **Results:** 30 pts have been treated (22 male, 8 female, age 26-83). 16 had cutaneous primary, 3 mucosal, 11 unknown primary. At enrollment, 16 had recurrent disease after prior surgery, 4 had in-transit disease. 6 received prior adjuvant therapy with ipilimumab (4) or HDI (2). 15 had stage IIIB, 10 IIIC, 5 stage IV (AJCC 7). >230 cycles have been delivered (median 16), and 29 pts have undergone definitive surgery to date. HDI was dose reduced in 18 pts, P discontinued in 8. Clinical data are available for 28 pts to date: radiologic preoperative RR was 75% (95% CI, 57-87) (6 CR, 15 PR). 21% (6) had SD and 1 had PD as best response at restaging scans before surgery. 4 pts recurred and 2 died. The pathologic complete response (pCR) of 26 pts was 31% (95% CI, 17-50). There is no significant difference in RR or pCR in pts with prior adjuvant therapy. Median f/u time is 17.37 mos, median PFS/OS not reached. Most common grade (Gr) 3 toxicities: fatigue (9; 32%), ↑CPK (6; 21%), ↓phos (9; 32%), ↑lipase (4; 14%). 3 Gr 4 events (CPK, hyperglycemia, lymphopenia). 1 suspected grade 5 event occurred 6 months after completion of therapy with autopsy evidence of pneumonia and myocarditis. **Conclusions:** Neoadjuvant P-HDI has promising clinical activity. Longer follow up is ongoing and correlative analyses are underway.

Outcomes for patients with metastatic uveal melanoma (MUM) treated with ipilimumab and nivolumab (cIN): a multi-center, retrospective study

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*YN, KN; ^PF, AS contributed equally **Introduction:** Uveal melanoma is the most common intraocular malignancy, and accounts for 3-5% of all melanomas. 50% of patients develop metastatic disease despite local treatment. Median survival for MUM is poor and there is no standard therapy. cIN is approved for cutaneous melanoma, but its efficacy in a large cohort of MUM is unknown. **Methods:** A multicenter, retrospective analysis of MUM patients treated with cIN included 66 pts from 11 U.S. centers. Progression free survival (PFS) and overall survival (OS) were estimated by Kaplan-Meier methodology. **Results:** Mean age at initiation of therapy was 58 years. 47% of pts were female (31), 53% male (35) and 65% (43) were Caucasian. 35% (23) had prior therapy (Rx) for MUM: 6 liver directed Rx, 3 radiation, 13 immuno Rx, 6 targeted Rx, 2 chemo Rx. Median number of cycles of cIN completed was 3. 65% (43) had progression of disease (PD) as best response, 17% (11) stable disease (SD), 11% (7) partial response (PR), 2% (1) had

complete response (CR), 6% (4) were inevaluable. Overall response rate (ORR, PR+ CR) was 13% (8/62). Clinical benefit rate (CBR, SD + PR + CR) was 31% (19/62). 58% [95% CI: 37-90%] of pts with clinical benefit had response for > 6 months (mo) with median follow-up of 8.5 mo. Median OS was 13.6 mo [95% CI: 10.9-27.3 mo], median PFS was 2.7 mo [95% CI: 2.5-3.7 mo]. Common toxicities were colitis (33%), transaminitis (21%), thyroiditis (20%), rash (18.2%). Uveitis in 2 pts, DKA, myositis, AKI in 1 each. Treatment was discontinued in 61% (40), most commonly for toxicity (48%; 19) or PD (35%; 14). **Conclusion:** To our knowledge, this is the largest reported cohort of cIN-treated MUM pts. In a 'real-world' setting, cIN for MUM has low efficacy and high rates of toxicity. Prospective trials are underway.

Phosphoglycerate dehydrogenase upregulation confers resistance to MEK inhibition in NRAS mutant melanoma

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Melanomas frequently harbor NRAS mutations but there are limited therapeutic options for this subset. One of the main pathways activated downstream of mutant NRAS is RAF-MEK-ERK1/2 signaling but inhibitors to this pathway are limited by resistance. Tumors re-wire metabolic pathways in response to stress such as targeted inhibitors but most resistant pre-clinical models are generated in conditions that lack physiological metabolism. We generated human mutant NRAS melanoma xenografts that were resistant to the MEK inhibitor (MEKi) PD0325901 in vivo. MEKi-resistant (MEKiR) cells showed cross-resistance to the structurally distinct MEKi, trametinib and elevated ERK1/2 phosphorylation and downstream signaling. Additionally, we observed frequent upregulation of phosphoglycerate dehydrogenase (PHGDH), which catalyzes the first reaction in the serine synthesis pathway. Suppressing PHGDH with siRNA knockdown and pharmacological inhibition, we observed a decrease in total glutathione production, the largest sink for reactive oxygen species in cells, and cell proliferation in both Ctl and MEKiR cell lines in the presence of PD901. This effect was greater in cell lines overexpressing PHGDH compared to those that do not. Our data suggests targeting the serine synthesis pathway as a potential strategy in overcoming MEKi resistance.

CD271 expression correlates with melanoma progress in case-control study

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Stem-like melanoma cells have been identified in mouse and *in vitro* models. Conceivably, they drive tumor progression rather than cells of the tumor bulk. Because elevated tumor-cell proliferation is an established indicator of aggressive disease, this study aimed to investigate the correlation between melanoma recurrence and proliferation of putative melanoma stem cells. Additionally, their expression in nevi, melanomas, and their recurrence was studied.Thirty patients with relapse (cases) and 30 without (controls) were matched for important prognostic markers. One section of the patients' primary melanoma (n=60), relapse (n=21), and nevus (n=17) were immunohistochemically double-stained for Ki67/MART1 and single-stained for CD271, CD166, and CD20. Their whole slide images were aligned as virtual quadruple stains. Image analysis established proliferation indices of each putative stem-cell marker and the tumor bulk in addition to the markers' percentage level in tumor areas and epidermis. In cases vs controls, median dermal proliferation indices (no./mm^2) were 211 vs 103 (P=0.04) for CD271, 512 vs 227 (P=0.3) for CD166, 184 vs 97 (P=0.3) for CD20, and 95 vs 103 (P=0.6) for the tumor bulk. Of additional interest, epidermal CD271$^+$keratinocytes added up to 8.8% in nevi and 0.98% in melanomas (P=0.0007). Even though differences between nevi and melanomas also were observed for CD166 in both epidermis (P=0.002) and dermis (P=0.006), they were visually less apparent. CD20$^+$MART1$^+$cells were absent in half of the melanomas, and all nevi and relapses. In conclusion, high levels of CD271$^+$Ki67$^+$MART1$^+$cells were linked to melanoma relapse as opposed to common Ki67 indices in this particular case-control study. Especially, loss of epidermal CD271$^+$keratinocytes seemed necessary for melanoma development; hence, identification may serve as a diagnostic tool with additional research.

The combination of PI3Ki and MEKi: a treatment option for BRAF mutant and BRAF WT patients?

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New therapy concepts, such as immunotherapy as well as targeted therapy, have significantly improved the overall survival of melanoma patients in recent years. However, about 20 % of patients do not respond to initial targeted therapy and at the same time, most of the tumours develop resistance through long-term therapy, e. g. by intensifying the activation of the PI3K-AKT-mTOR signalling pathway. Clinical studies in breast cancer show that both the pan-PI3K inhibitor BKM120 and the PI3Kα-selective inhibitor BYL719 have antitumour activity. This raises the question of whether these inhibitors are also a therapeutic option for melanoma and whether a combination with MEK inhibitors could further restrict growth and prevent possible development of resistance. At the same time, it will be investigated which mutations of the tumor indicate a positive therapeutic result.

BRAF and NRAS mutant cell lines, as well as direct isolated patient tumor cells are treated with the PI3K inhibitors BKM120 and BYL719 and in combination with the MEK inhibitor trametinib. In addition to the investigation of cell cytotoxicity and cell cycle

remainder, the altered signal transmission is detected. Furthermore, the patient cells are sequenced in order to identify mutations that promote a positive therapeutic response.

While the pan-PI3K inhibitor BKM120 is cytotoxic in almost all cell lines and patient cells, the PI3Kα-selective inhibitor BYL719 does not have an antitumour effect. However, the combination of the PI3K inhibitors with the MEK inhibitor already shows a significantly stronger cytotoxic effect at lower concentrations compared to monotherapies.

These data show that the combination of PI3K inhibitors with MEK inhibitors could be a new therapeutic option for melanoma patients. By using PI3Kα-selective inhibitors, potential side effects could be reduced compared to pan-PI3K inhibitors.

Mutated MITF-E87R in Melanoma Enhances Tumor Progression via S100A4

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Melanoma, a melanocyte origin neoplasm, is the most lethal type of skin cancer and incidence is increasing. Several familial and somatic mutations have been identified in the gene encoding the melanocyte lineage master regulator, microphthalmia-associated transcription factor (MITF); however, the neoplastic mechanisms of these mutant MITF variants are mostly unknown. Here, by performing unbiased analysis of the transcriptomes in cells expressing mutant MITF, we identified calcium-binding protein S100A4, as a downstream target of MITF-E87R. By using wild-type and mutant MITF melanoma lines, we found that both endogenous wild-type and MITF-E87R variants occupy the S100A4 promotor. Remarkably, whereas wild-type MITF represses S100A4 expression, MITF-E87R activates its transcription. The opposite effects of wild-type and mutant MITF result in opposing cellular phenotypes, as MITF-E87R via S100A4 enhanced invasion and reduced adhesion in contrast to wild-type MITF activity. Finally we found that melanoma patients with altered S100A4 expression have poor prognosis. These data show that a change in MITF transcriptional activity from repression to activation of S100A4 that result from a point mutation in MITF alters melanoma invasive ability. These data suggest new opportunities for diagnosis and treatment of metastatic melanoma.

Myosin II reactivation and cytoskeletal remodelling as a hallmark and a vulnerability in melanoma therapy resistance

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Targeted and immunotherapies improve survival of a proportion of melanoma patients. However, lack of response or therapy resistance are persistent problems in melanoma management.

In this study, we provide evidence that early adaptation to treatment and acquisition of resistance to MAPK inhibitors (MAPKi) involve profound cytoskeletal remodelling. This is concomitant with changes at the transcriptomic and phospho-proteomic level of many cytoskeletal proteins. Most significant changes are observed in the ROCK-Myosin II pathway, widely studied for its key role in cancer invasion and metastasis. Furthermore, we find that MAPK signalling itself positively controls Myosin II activity. After initial therapy response, drug-resistant clones restore MAPK and, in turn, Myosin II levels by increasing expression of myosin light chain 2 (MLC2), ROCK, LIM kinase (LIMK) and myocardin-related transcription factor (MRTF).

Cross-resistance to MAPKi and immune checkpoint inhibitors has been recently proposed to be controlled by common transcriptomic alterations. We report that high ROCK-Myosin II activity and its characteristic transcriptome identify both targeted- and immunotherapy-resistant melanomas. Importantly, resistant cells are more dependent on Myosin II activity for their survival. ROCK1/2 or Myosin II activity blockade with either small molecule inhibitors or RNAi depletion induces death of resistant cells through a combination of cell cycle defects and decreased survival signals. Furthermore, efficacy of both targeted and immunotherapies can be greatly improved by combining such therapies with ROCK inhibitors *in vivo*.

Therefore, we propose that a subset of targeted and immunotherapy-resistant melanomas is more vulnerable to ROCK-Myosin II inhibition, which opens up clinical opportunities for combination therapies.

Immune Co-Culture Cell Microarray - a Feasible Tool for Investigating Immunotherapy-related Mechanisms in a High Throughput Manner

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In order to examine the role of potential immune targets, their expression must be assessed over time in a functional immunological assay. However, due to inter-experiment variations, standard in-vitro assays cannot properly assess dynamics in the expression of multiple targets. Our aim was to develop a feasible tool for high-throughput assessments of expression dynamics during a lymphocyte-cancer cell killing assay. Two autologous pairs of tumor infiltrating lymphocytes and melanoma which were extracted from patients were co-cultured and later harvested at five time-points. Co-culture from each time-point was turn into a cell block, and all blocks were turn into a microarray. Protein expression was assessed by immunohistochemical (IHC) and immunofluorescence (IF) stains as well as by flow cytometry (FC). The immunogenic cell death marker activated Caspase-3 had similar expression pattern overtime among IHC, IF and FC, demonstrating that the microarray properly represents immunological activity. IHC and IF stains of the immune checkpoints CEACAM1 and PDL-1 demonstrated different dynamics – CEACAM1 was over-expressed in ~50% of the melanoma cells early in the killing process, while PDL-1 expression was over-expressed only late in the killing process but in all living melanoma cells. In conclusion, the ICCM is a feasible tool that properly represent immunological lymphocyte-melanoma activity. Since each ICCM slide represent the same experiment, multiple consecutive stains can be used to compare the dynamic in expression of many possible targets, while avoiding inter-experiments variations.

Real world data of use and sequence of targeted and immuno-oncology drugs in Metastatic Melanoma (MM) patients (pts)

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Data on of optimal sequence of immunotherapy (iTx) or BRAF targeted therapy (tTx) are scant. Our aim was to characterize and compare the outcomes of MM pts treated in real-world setting, identify factors of clinical benefit (CB). Single-institution experience of 115 consecutive pts with MM treated in our center from 2010 to 2017. Clinicopathological factors (CPF), treatment (Tx) pattern and survival were analyzed. CB was defined as complete, partial response, or stable disease more than four months (m). Median (Md) age was 62y. BRAF-mt was 46%. Md follow-up was 24 m. 1[o]-line (1L) Tx included 40% iTx, 43% tTx and 16% others Tx (oTx). 2[o]-line (2L) Tx were 77% iTx, 16% tTx and 7% oTx. At 3[o]-line (3L) iTx was most usual 80%. Md overall survival (OS) was 27 m. Md Time to Tx failure (TTF) was 3.7 m. Of the total of 222 Tx, TTF was 5.3 m for tTx, 3.3 m for iTx and 2.6 m for oTx. CB was higher with tTx 63% than iTx 46% or oTx 25% (p<0.01). Toxicity grade 2+ was higher for tTx 65% than iTx 56% or oTx 27%, (p<0.04). For BRAF-mt pts, 87% received tTx at 1L and 54% received additional Tx, the sequence of tTx followed by iTx was more common. Regards to iTx, TTF was not influenced by Tx line (p>0.05 all comparisons). According to iTx regimen, anti-PD1/L1 had longer TTF respect to anti-CTLA4 (HR 0.62 p=0.04); CB was lower at more advanced lines reaching 61% at 1L, 40% in 2L and 35% in 3L. 5 pts received chemotherapy after iTx, one of had CB. In pts receiving iTx with previous iTx, TTF was 3.3 m and CB was 34%. In multivariable analysis, CPF did not impact on OS but pts with LDH ≥1.5 ULN at 1L had a trend for worse outcomes (HR 2.1 p=0.17).Our real-world data confirms long-term OS in MM pts exposed to tTx and iTx. Benefit of iTx is seen in all lines. Our tendency in BRAF-mt was tTx follow of iTx. Additional research is needed to define prognostic markers in this pts.

Delayed severe colitis with anti-PD1 based therapy

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Colitis is a common irAE with combination anti-PD1-/CTLA4- (combi) immune therapy, and is associated with anti-PD1-monotherapy (PD1 mono) although less frequent and usually less severe. Colitis most often occurs within 12 wks for combi therapy. The timing of anti-PD1 colitis is varied and may be delayed, even beyond cessation of therapy in selected case reports. In this retrospective analysis, cases of grade (G) 3/4 colitis with PD1 mono or combi were reviewed to investigate timing in relation to immune therapy. Demographics and disease characteristics were recorded and cases were examined for timing of onset, severity and treatment.

There were 47 cases of grade 3/4 colitis since 2013; 6 with PD-1 mono and 34 with combi therapy (in 7 pts remain blinded to nivo or ipi/nivo). 5 resulted in colectomy. 4 cases occurred >1yr after commencement (at 13mo, 14mo, 3yr and 4yr 2mo); all were associated with PD1 mono therapy and in 3 cases PD1 was ceased prior to onset of colitis. 2 cases occurred after completion of 1yr adjuvant

therapy (at 13mo and 14mo), one G3 treated with IV methylprednisolone and one G4 requiring colectomy. One pt had 2yr pembrolizumab for metastatic melanoma with good partial response, and later developed G4 colitis requiring colectomy 2yr 2mo after cessation of treatment. One pt had G3 colitis after 3yr of nivolumab. There were 2 cases of G3/4 PD1 mono therapy colitis occurring <5wks of commencement. In the combi group, there were 31 G3 and 3 G4 events. Timing of onset was <5 wks in 14 cases (41%), 5-15 wks in 16 cases (47%), and 15-21 wks in 4 cases (12%). In total 12 cases were related to (neo)adjuvant therapy, and 35 related to metastatic therapy.

Timing of severe PD1 mono colitis is unpredictable, and may be delayed. Further work is required to understand the pathophysiology in these cases. Patients and clinicians should be aware that delayed toxicity can present years after treatment, albeit rarely.

Multiplex germline testing of melanoma cases in Latvia suspected to genetic predisposition
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Cutaneous melanoma is the most aggressive skin cancer that accounts for the majority of skin cancer related deaths. In approximately 10% of melanoma cases, this malignant disease occurs in family setting. During the last two decades, several high-risk genes have been identified and include *CDKN2A*, *CDK4*, *BAP1*, *TERT*, *POT1*, *ACD*, *TERF2IP,* and *POLE*. We performed genetic analysis using a custom-made high melanoma risk genes (*CDKN2A*, *CDK4*, *BAP1*, *POT1*, *TERT*) panel based on HaloPlex[TM] NGS library preparation method for Ion Torrent sequencing in Latvian melanoma patients with an evaluated disease risk. Altogether, 108 primary melanoma patients including those with family history of melanoma (n=33), patients with an early age of disease onset (<40 years) (n=67), patients with MPM (n=6) as well as patients with rare uveal melanoma (n=2) were analysed.

We confirmed *CDK4* (R24H) as a main risk gene in Latvian families with a strong family history of melanoma. A novel non-sense *POT1* variant p.Leu153* was detected in melanoma patient with multiple other cancer cases in the family. A novel *POT1* splice donor site variant c.546+1G>A was found in a young (28 years) melanoma patient with lung and breast cancer cases in the family. Previously reported *POT1* variant p.Gly404Val was detected in a melanoma patient with pancreas cancer in the family. Seven melanoma patients, below age 40 years, had *TERT* variants p.Ala279Thr (n=4) and p.Ala1062Thr (n=2), that previously have been associated with oesophageal, lung cancer and leukaemia, as well as rare variant p.His815Arg (n=1). A rare *BAP1* variant p.Pro293Leu was discovered in one of the uveal melanoma patients. Resequencing using NGS approach has led to the discovery of several novel and functionally significant variants associated with inherited melanoma in Latvia and has to be proven appropriate for screening patients at increased disease risk.

Evidence of intussusceptive angiogenesis in human melanoma metastases
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Angiogenesis, formation of new blood vessels, is required to provide oxygen and nutrients to growing tissues as well as growing tumors. There are two forms of angiogenesis; sprouting and intussusceptive. In sprouting angiogenesis (SA), pre-existing vessels send *tip cells* to follow angiogenic stimulants such as VEGF, to form new vessel branches. SA is well characterized and several drugs targeting VEGF have been produced. Sadly, the clinical effects of these drugs have been weak or absent in melanoma. Intussusceptive angiogenesis (IA), a form of neovascularization less studied, causes a vessel to splits longitudinally into two parallel vessels, and seems to be independent of VEGF. IA is a fast and efficient way of rapidly expanding and remodeling vascular beds, and its role in development is well established. However, the role of IA is still to be understood in the growth of human tumors. Therefore, we sought to investigate if IA is present in human melanoma metastases.

We analyzed human melanoma metastases (n=6) for signs of IA. The aim was to detect transluminal *pillars*, the histological hallmark structure of IA. Pillars are thin endothelial bridges (2-4 μm in diameter) which mature as collagen fibers and perivascular cells invade their core. Consecutive pillars fuse and cause the formation of a vessel wall separating two lumens. Since pillars are small 3D structures they are not detected by routine microscopy. Therefore, we used 3D high resolution imaging techniques to search for them. Applying our approach in human melanoma metastases, we detected typical pillars in different stages of maturity and with striking resemblance to intussusceptive pillars demonstrated in development.

In conclusion, we provide evidence of intussusceptive angiogenesis in human melanoma metastases. This may be the missing puzzle piece explaining the poor clinical effect of angiogenic inhibitors in melanoma up until now.

Cutaneous toxicities with anti-PD1 monotherapy: incidence, management, and outcomes in the real-world setting
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Anti-PD1 (PD1) therapy has rapidly evolved into a mainstay for metastatic melanoma (MM). From clinical trial data, approximately 40% of patients treated with PD1 will have a cutaneous adverse event (CAE), most commonly rash or vitiligo; and additional cohort studies indicate a correlation between CAE and improved progression-free survival. We conducted a retrospective chart review of consecutive patients treated with PD1 monotherapy in 2015 (N=278).

The incidence of CAE in our cohort was 13%, and patients with and without CAE were similar sex, baseline LDH, age at treatment onset, and had the same total number of MM treatments. The most common CAEs were eczema (21%) and vitiligo (18%) followed by acneiform eruption (8%). PD1 dosing was impacted in six (17%) pts with CAEs, or 2% of overall treated patients. CAEs had a high rate of resolution with 32 (80%) either resolved, or stable and asymptomatic. The median time to onset of all CAEs was 10.4 weeks, with the 7 vitiligo patients having a longer median time to onset of 38 weeks. Median rash duration was 21.6 weeks. The long duration of CAE, high rate of CAE resolution, and low incidence of dose impact define mainly low-grade CAEs. Time to next treatment, calculated as the interval from PD1 start to the start of the next treatment or last follow-up, was significantly longer for patients with CAE (median not reached), compared with patients without CAE (median=246 days, p<0.001).

In conclusion, we observed an incidence of 13% CAEs in our consecutive cohort of 278 pts treated with PD1 in 2015. The lower incidence, compared with that observed in clinical trials, is likely due to decreased toxicity monitoring and documentation in the real-world setting. PD1 CAEs are low-grade, and have a low impact on the ability to remain on treatment, while their presence is associated with prolonged clinical benefit.

Cutaneous toxicities in immune checkpoint inhibitor combination therapy: features, management, and outcomes

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Immune checkpoint inhibitors (ICIs) have rapidly become the standard of care for metastatic melanoma (MM) patients (pts), and combination therapy with anti-CTLA4 and anti-PD1 agents (COMBO) is being used more frequently. Clinical trials of ICI monotherapy to treat MM had cutaneous adverse event (CAE) rates of 40-50%, but there is limited literature describing the CAE profiles of COMBO. A retrospective chart review was performed to describe CAEs with COMBO for treatment of MM.

A total of 155 pts started 157 distinct regimens of COMBO from 2012-2017. There were 94 CAEs attributed to 82 (52%) regimens. Only 59 (38%) regimens completed the combination (anti-CTLA4 + anti-PD1) phase, and continued on to the anti-PD1 monotherapy phase. Of the 98 regimens that were stopped early, 59 (60%) stopped due to toxicities; 4 of which had CAEs, and the remainder had diarrhea, colitis, or endocrinopathies. Overall, 8% of pts had an impact on their COMBO dosing because of CAEs, including dose delays.

The most common CAEs were eczema (26%), morbilliform rash (23%) and vitiligo (12%). Median time to onset of CAE was 3 weeks, and median duration was 8 weeks, normally clearing after the pt started the anti-PD1 phase. Only 12 (13%) of the CAEs required systemic steroids, and the majority of pts (89%) had rash resolution, or were stable/asymptomatic.

No significant differences were observed between pts at COMBO start with respect to age, sex, and LDH, however, the interval from starting COMBO to starting a new treatment for MM was significantly longer for pts that developed a CAE (median=448 days), compared with pts that did not develop a CAE (median=168 days, p=0.001).

This is the first in-depth, real-world evidence of CAEs attributed to COMBO, which had high toxicity and high CAE incidence (52%). The CAEs were, however, low grade, and associated with a prolonged clinical benefit.

Percutaneous Oncolytic Rose Bengal Disodium for Metastatic Uveal Melanoma Patients with Hepatic Metastases

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Rose bengal disodium (PV-10) is a small molecule oncolytic immunotherapy in clinical development for treatment of solid tumors. When administered by intralesional injection, PV-10 can produce an immunogenic cell death that may induce a T-cell mediated immune response against treatment-refractory and immunologically-cold tumors. Given this mechanism of action, we investigated treatment of metastatic uveal melanoma with percutaneous hepatic PV-10.

PV-10-LC-01 (NCT00986661) is an open-label Phase 1 study evaluating the safety, tolerability, and preliminary efficacy of intralesional PV-10 in patients with solid tumors metastatic to the liver. A single percutaneous injection of PV-10 is administered to a designated hepatic tumor 1.0-4.9 cm in diameter. Response assessments are performed at Day 28, then every 3 months. Patients with

multiple injectable tumors may receive further PV-10 after Day 28.

PV-10-LC-01 includes a single-center cohort of 10 uveal melanoma patients with hepatic metastases. Eligible patients may receive standard of care checkpoint blockade immunotherapy during treatment with PV-10.

To date, the study has screened 7 patients with metastatic uveal melanoma. Three patients have been consented, enrolled, and treated; one patient has received a second treatment with intralesional hepatic PV-10. Two patients have received PV-10 with standard of care immunotherapy. Updated enrollment as well as preliminary safety and tolerability of the uveal melanoma cohort will be presented at the meeting.

Systemic adjuvant melanoma treatment: Implications for UK melanoma services

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For patients with completely resected stage III melanoma, surveillance is the current UK standard of care. Following recent positive clinical trial results, NICE decisions for systemic adjuvant melanoma treatments (nivolumab/dabrafenib+trametinib) are expected by 2019; this represents a significant change in patient management affecting the whole Specialist Skin Cancer Multidisciplinary Team (SSMDT).
In April-May 2018 we conducted 49 structured interviews with UK health professionals involved in melanoma management (28 oncology, 12 dermatology, 9 surgery) from 34 geographically-dispersed NHS Trusts (1-6 respondents/Trust). Objective: to identify current management, expected changes when adjuvant treatments are available and implications for UK melanoma services. Respondents expect to see a median of 5 (1-35) patients/month who will be eligible for adjuvant therapy. Currently, only 31 (63%) respondents include standardised BRAFV600 mutation testing for primary melanoma in their local guidelines. 30 (61%) respondents are from centres offering sentinel lymph node biopsy (SLNB) on-site after excision of melanoma from the trunk/limbs; 21 (43%) after excision of head/neck melanoma. Referral for SLNB (if not carried out on-site) is not always offered. When systemic adjuvants are available, respondents expect oncology involvement in patient care to increase considerably. Significant impacts are predicted on staffing (39, 80%), training (32, 65%), commissioning (23, 47%), service structure (38, 78%), local guidelines (40, 82%) and psychological support for patients (30, 61%).
The introduction of systemic adjuvant melanoma treatments has broad implications for melanoma service organisation and delivery. To ensure equitable and efficient patient access, UK SSMDTs may need to review their current melanoma service provision and implement the guidelines and infrastructure required to deliver adjuvant therapy.

Immunotherapy or targeted therapy as initial therapy for advanced melanoma: a real-world analysis

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The initial management of patients (pts) with advanced stage *BRAF*-mutated melanoma includes immuno-oncology (IO) and targeted (TT) therapy. An ongoing randomized trial is comparing these therapies (NCT02224781); real-world analysis may provide early insights. Electronic health records from 15 cancer centers (Cota observational database) were reviewed to identify pts with *BRAF* V600-mutated melanoma (excluding pts with prior adjuvant therapy or in a clinical trial). 104 *BRAF*-mutated pts received first-line therapy for metastatic (n=101) or unresectable (n=3) melanoma between Nov 2011-Nov 2017. Median age was 66 yr (61% male); 48 pts received IO and 56 TT. Median follow-up was 11 mo (range 1-61; IO=16 mo, TT=10 mo). Pts treated with IO and TT were similar in age, race, sex, academic and community setting, presence of symptoms, number of mets sites, and presence of brain mets. More pts on TT had liver mets (IO 17% vs TT 38%, p=0.03) and elevated LDH (IO 35% vs TT 57%, p=0.02). Median Kaplan-Meier estimated overall survival (OS) was higher with IO (IO=23 mo, TT=14 mo; log-rank p=0.03), with 1-yr survival rates of 76% for IO and 50% for TT. Among pts with low-risk characteristics (absence of symptoms p<0.01, no liver/brain mets p=0.02, <2 sites of mets p=0.04, normal LDH p=0.09), pts on IO had significantly improved OS compared with TT; no significant difference was found in pts with high-risk characteristics. After adjusting for differences in pt characteristics, pts on IO had a 47% reduced risk of death compared with TT (HR 0.53; p=0.03). This real-world analysis of pts with advanced stage *BRAF*-mutated melanoma provides an early insight that initial therapy with IO may result in improved OS. However, this retrospective study cannot control for time-related differences in drug availability and undefined variables of pt risk. Accrual to a randomized trial remains a priority.

Clinical observational study of talimogene laherparepvec (T-VEC) use among melanoma patients (pts) in routine clinical practice in the United States (COSMUS)

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Advanced locoregional melanoma poses a significant therapeutic challenge. T-VEC, a modified oncolytic herpes virus, is an intralesional therapy for unresectable advanced melanoma. A chart review of 76 melanoma pts treated with T-VEC (first dose 27Nov2015-15Dec2016) at 7 academic US sites analyzed demographics, clinicopathologic characteristics, outcomes, and adverse events. Median age was 73 yrs (range 30-93); 45 pts (59.2%) were male. Stage at T-VEC start: 42 (55.3%) IIIB-IVM1a, 30 (39.5%) IVM1b-IVM1c, and 4 (5.3%) unknown. Median number of administrations was 6 (range 1-19). Checkpoint inhibitors (CPI) were used in 30 (39.5%) pts before T-VEC, 11 (14.5%) after T-VEC, and 10 (13.2%) with T-VEC. Eight pts (10.5%) remained on T-VEC by study end, 14 (22.1%) had no injectable lesions left (10 T-VEC only, 3 CPI before T-VEC), 1 had a pathologic complete response (CPI and T-VEC), and 53 (77.9%) pts discontinued T-VEC (29 pts due to disease progression). In pts with no remaining injectable lesions including 1 complete response, median duration of therapy was 3.0 (range 1.6-9.0) months (mos). For pts still on therapy, median duration of therapy was 8.9 mos and for those who progressed, median duration of therapy was 2.1 mos. Twenty pts (26.3%) died, 13 from disease progression. By the end of data collection (median follow-up 9.4 mos, range 0.1-17.1), overall survival was 78.6% in stage IIIB-IVM1a and 63.3% in stage IVM1b-IVM1c pts. Adverse events of interest were reported in 21 pts (27.6%): flu-like symptoms were most common (n=8, 10.5%) followed by lesion ulceration (n=4, 5.3%). No herpetic infections were reported. In summary, T-VEC was well tolerated and showed clinical utility in this cohort of pts with unresectable advanced melanoma, which was older and more heavily pretreated than that in the OPTiM trial.

The RNA helicase DDX3X dictates translation re-programming and drives therapy resistance in melanoma
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In melanoma, persistent formation of translation initiation complexes and synthesis of selective mRNAs under stress promote phenotypic plasticity that is linked to drug resistance and metastatic properties.
Here we found the RNA-helicase DDX3X to be significantly mutated in melanoma tumors. Patients with DDX3X-low tumors displayed significantly worse relapse-free survival than patients with DDX3X-high tumors. To study the impact of *DDX3X* loss in melanoma, we employed siRNA-mediated *DDX3X* knockdown and showed that global protein biosynthesis was considerably reduced. Moreover, *DDX3X* downregulation also promoted elevated migration capacity. We performed a genome-wide screening and sequenced transcripts associated with translationally active ribosomes isolated from siDDX3X and siCtrl HT144 cells. Interestingly, *MITF* was amongst the most translationally repressed transcripts in *DDX3X*-depleted cells. By subcloning the human MITF 5'UTR that contains energetically favorable RNA secondary structures, into a translational reporter, we identified a stem loop (SL) element to be required for DDX3X-dependent regulation of *MITF* translation. Further, deleting the SL sequence from the endogenous MITF 5'UTR in melanoma cells using CRISPR/Cas9, we found that ΔSL MITF cells in vivo developed significantly smaller tumors compared to controls and showed significantly more micrometastases. Moreover, knockdown of *DDX3X* induced BRAFi treatment resistance. Upon BRAFi treatment *DDX3X* was transcriptionally down-regulated while *MITF* was translationally inactivated through its 5'UTR. In support, we observed drastic reduction of *DDX3X* mRNA levels in human biopsies from BRAFi resistant patients.
In summary, we propose that DDX3X-mediated translational control represents an efficient way to rapidly modulate MITF abundance which has a crucial role in melanoma progression and therapy response.

Melanoma liver metastases (mets) are immunosuppressive and have a distinct clinical and genetic profile.
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Melanoma patients (pts) with liver mets have a lower response rate (RR), & shorter progression-free (PFS) & overall survival (OS) vs pts without liver mets when treated with anti-PD-1 (PD1) therapy. To explore this further we compared clinicopathological features,

circulating cytokines & tumor gene expression profiles of pts with & without liver mets treated with PD1 combined with ipilimumab (PD1+IPI).

Demographics, disease characteristics, white cell subsets & outcome data were collected in pts treated with PD1+IPI (n=140). Circulating cytokines & tumor gene expression data were compared between pts with & without liver mets.

Compared with pts without liver mets (n=101), pts with liver mets (n=39) had a lower RR (44% v 75%), and shorter med. PFS (4.14 months vs not reached) & OS (p<0.05) to PD1+IPI. In contrast, in a control group of pts treated with BRAF targeted therapy, RR, PFS & OS were similar between pts with (n=19) vs without (n=57) liver mets. In a multivariate analysis performed on the PD1+IPI cohort & validated in the control group, higher ECOG (OR 4.3 p=0.003), presence of bone (OR 4.6 p=0.004) & spleen mets (OR 13.5 p=0.01) & higher monocyte count (OR 4.1 p=0.01) were associated with the presence of liver mets. The expression of 65 cytokines was measured in plasma of treatment-naive pts, & pts with liver mets (n=35) had higher levels of Eotaxin 2 (p=0.01), IP-10 (p=0.02) & IL-18 (p=0.04) vs pts without liver mets (n=105). Gene expression analysis of melanoma samples showed higher expression of MMP-8 & HIF1a in pts with (n=58) vs without (n=28) liver mets, & was validated in an independent cohort (n=58).

Pts with liver mets display a distinct disease characteristics & melanoma gene expression profile, & are less responsive to PD1+IPI. Liver mets microenvironment may hold unique immunosuppressive mechanisms that are amenable to therapeutic targeting.

Haematologic predictors of response & toxicity (tox) in metastatic melanoma (MM) patients (pts) treated with anti-PD-1 alone (PD1) or with ipilimumab (IPI+PD1)

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Several blood factors have been proposed to be prognostic in MM pts treated with PD1 or IPI. A low lymphocyte (lymph), high neutrophil (neut) count & high neut/lymph ratio (NLR) at baseline (BL) associate with shorter overall survival (OS). Increases in lymph count 2 to 8 weeks after IPI correlate with longer OS. We sought to examine clinical & blood factors that associate with outcomes (ORR/PFS/OS) or tox (overall & specific) with PD1 or IPI+PD1.

Two cohorts were examined, 343 PD1 pts & 140 IPI+PD1 pts with MM. Demographics, disease characteristics, blood factors (BL & early during treatment [EDT] - weeks 3 & 6), outcomes (ORR/PFS/OS) & tox were examined.

In multivariate analysis, elevated LDH at all timepoints was associated with inferior ORR/PFS/OS for both treatments. In PD1 pts, higher monocyte (mono) count at BL & at 3 weeks associated with inferior ORR/PFS/OS & a higher lymph count at week 6 associated with superior ORR/PFS/OS. Higher NLR at BL & week 3 was associated with shorter OS, but not with ORR or PFS in PD1 pts.

No association was found between clinical/blood factors & tox in IPI+PD1 pts, beside older age & rash (OR 2.5, p=0.02). In PD1 pts, older age (OR 1.8, p=0.03) & higher haemoglobin (Hb; OR 6.6, p<0.001) at BL associated with overall tox. In PD1 pts, higher Hb at BL associated with rash, colitis, hepatitis & thyroiditis. Female sex & higher eosinophil count at baseline associated with thyroiditis. No associations were found with other specific tox with either treatments.

In multivariate analyses incorporating clinical & haematologic factors, LDH is a strong predictive marker for response with both PD1 & IPI+PD1 therapy, while NLR is prognostic for OS in PD1 pts. Further factors, such as mono & lymph counts at BL & EDT may also be predictive of response in PD1 pts.

MYC and NF-kB signalling play a role in shaping the immune response to melanoma.

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Bioinformatic analysis of 703 primary melanoma transcriptomes (from the Leeds Melanoma Cohort) was applied to characterise subgroups with differing immune microenvironments. Using immune-cell scores, consensus clustering identified 3 immune subgroups with transcriptomic patterns: low, intermediate and high immune. The differentially expressed genes were identified using standard statistical methods and analysed in Cytoscape using Reactome FIVIZ and Centiscape plugins.

The nodal gene for the low immune subgroup was *MYC* and for the high immune *NFKB1*; both were then correlated with protein scores from immunohistochemistry. We also examined copy number variations (CNVs) at those genes and NF-κB and IFN-γ signalling genes across the immune subgroups. We observed amplifications of *MYC* in 29% and deletions of *NFKB1* in 14% of the low immune tumours, which was more than in the intermediate (*MYC*: 20%, *NFKB1*: 2%) or the high immune subgroups (*MYC*: 11%, *NFKB1*: 0%): P= 0.02 for *MYC*, P=0.0003 for *NFKB1*. CNVs were strong predictors of prognosis (yes vs no, adjusted for AJCC stage) separately (*MYC* amplifications: HR=1.8, P=0.006; *NFKB1* deletions: HR=1.5, P=0.007) and when combined: HR=3.7, P=0.002. We found similar results for deletions of *JAK2, NFKB2, CHUK, MAP3K7, IRAK2,* and *MYD88*.

By testing *MYC* correlations in the melanoma cell line transcriptomes (with no immune cells) we showed that tumour-derived *MYC* was negatively correlated with many antigen processing and presentation genes (*HLA-C, B2M, TAP1 and ERAP1*), with *HLA-B* having

the strongest result: R=-0.57. In summary, we report deletion of genes involved in NF-κB signalling and amplification of *MYC* are common in primary melanomas with low immune cell infiltration. Loss of *JAK2* has been previously reported in relation to secondary resistance to checkpoint blockade, and our data suggest that similar genetic changes are present in primary tumours.

Detection of cell-free circulating *BRAF*[V600E] by droplet digital PCR in non-melanoma patients: considerations for the clinical implementation.

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Introduction: The analysis of cell-free *BRAF*[V600E] mutation (cf*BRAF*[V600E]) in plasma has emerged as a biomarker for monitoring prognosis and treatment response in *BRAF*[V600E] melanoma patients. However, the *BRAF*[V600E] alteration is a common event found in benign proliferations such as melanocytic nevi. We quantified the cf*BRAF*[V600E] in plasma from non-melanoma individuals under dermatological surveillance and melanoma patients to assess the clinical significance of the test.

We quantified the cf*BRAF*[V600E] in plasma from 146 non-melanoma individuals, 32 disease-free melanoma patients and 33 metastatic melanoma patients by droplet digital PCR.

Results: cf*BRAF*[V600E] was detected in 71.4% of stage IV and 15.4% of stage III melanoma patients. In addition, cf*BRAF*[V600E] was detected in plasma from 4.1% of individuals without melanoma at the moment of the plasma extraction. The cf*BRAF*[V600E] levels tend to be lower in non-melanoma patients compared to melanoma patients. The optimal cut-off value for identifying melanoma patients with >99% of specificity was a variant allelic frequency of 0.44% or 10 cf*BRAF*[V600E] copies/mL plasma. Interestingly, higher cf*BRAF*[V600E] levels were observed in individuals with a high nevus count.

Conclusion: The quantification of cf*BRAF*[V600E] has a strong clinical diagnostic value but detectable levels of the mutation in plasma are present in a subset of individuals without melanoma. Establishment of cf*BRAF*[V600E] positivity thresholds is required prior to the implementation of the test in the clinical setting.

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Molecular profiling of primary cutaneous melanomas reveals genes predisposing to brain metastases

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Brain metastases commonly occur in patients with advanced melanoma. Although targeted therapy and immune checkpoint inhibitors can benefit a subset of patients, the management of brain metastases remains a key unmet need. There are currently no validated clinical, pathologic or molecular factors that predict relapse to the brain. We present data from 1343 adults with stage IIB – IIIC (AJCC 7[th]Edition) primary cutaneous melanomas from a UK-based multicentre prospective clinical trial. Mutation panel and gene expression data from a subset of cutaneous primaries and regional lymph nodes were correlated with the first anatomical site of distant relapse with a minimum of 6 years follow-up.

We found no significant differences in the clinico-pathologic characteristics between primary tumours that relapsed to the brain versus those that relapsed to distant extracranial sites. There were no differences between these metastatic groups in terms of mutations in a select panel of driver genes, including genes implicated in the MAPK and PI3K signalling pathways. Differential expression revealed that selective genes involved in the neural crest and axonal-signalling pathways were differentially expressed in primaries that relapsed to the brain versus extracranial and these genes were not implicated in other site-specific comparisons.

The molecular pathogenesis of melanoma metastasis has previously been linked with signalling pathways in the embryonic neural crest. We hypothesise that particular neural crest induction and migration signals may have specific correlates with intracranial invasion.

Co-operation of *NF1* loss of function and non-V600 *BRAF* mutations in cutaneous melanoma

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Past studies have characterized a subset of BRAF non-V600 mutants that require additional cellular dysfunction that increase RAS to activate the MAPK pathway. This class of non-V600 BRAF mutations (Class III) are thought to be resistant to BRAF inhibitor targeted therapies due to their dimer-dependent signalling (Yao *et al.,* 2017). Several studies have also shown that loss of *NF1* in melanoma lines increases RAS activity and B-RAF activation, resulting in MEK1/2 and ERK1/2 phosphorylation (Whittaker *et al*., 2013; Nissan *et al.*, 2014). The TCGA and previous studies have reported that *NF1* loss of function mutations are anti-correlated with canonical, *BRAF* p.V600 mutations, but co-occur with non-p.V600 *BRAF* (Class III) mutations in melanoma. Based on past work, we hypothesized that loss of function mutations in *NF1* activates Class III *BRAF* mutants by promoting RAF dimerization. We overexpressed *BRAF* canonical/non-canonical mutants in NF1 knockdown cell lines. Expression of Class III BRAF mutants that were enriched with NF1 loss of function mutations reported in the TCGA dataset led to increased phospho-ERK activation upon NF1 knockdown. Interestingly, we observed an increase in homodimer formation when expressing *BRAF* wild-type and *BRAF* p.V600E, but not Class III *BRAF* mutants in cells with NF1 knockdown. We found that although Class III *BRAF* mutants bind wild-type CRAF more avidly than wild-type BRAF, Class III BRAF mutant/wild-type CRAF heterodimers are not increased when *NF1* is knocked-down. We speculate from our preliminary data that the co-ooperativity of Class III BRAF mutants and *NF1* loss may be due to an alternative mechanism not mediated by increased BRAF mutant/wild-type CRAF heterodimerization. Future studies will investigate this mechanism and test optimal MAPK inhibitor therapies to target melanomas with co-occurring *NF1* loss of function and non-p.V600 *BRAF* mutations.

Treatment Patterns of Melanoma by *BRAF* in the US in 2011-2017: a retrospective cohort study

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Melanoma treatment changes after introduction of new therapies since 2011 have not been studied well, especially in $BRAF^{Mut}$ melanoma. We studied 4197 melanoma patients who received systemic therapy (2011-17) in the US electronic medical record database OSCER, including 1687 with biomarkers (2011-16). Therapies included 64% checkpoint inhibitors (CPI), 19% BRAF/MEK inhibitors (BRAFi), 17% chemotherapy, 16% cytokines, and 1% oncolytic viral therapy. Overall CPI use grew from 23% to 81% (pembrolizumab 32%, nivolumab 23%, ipilimumab/nivolumab 21%) but ipilimumab use fell to 13%. Cytokine (43% to 3%) and chemotherapy (35% to 7%) use declined. Over 2011-17, CPI and BRAFi were used more in lines of therapy (LOT) 1-4 than as adjuvant, while cytokines were used as adjuvant only (64%). CPI were used more in $NRAS^{Mut}$ (85%) than $BRAF^{Mut}$, $BRAF^{wt}$, $NRAS^{wt}$ (57-66%). In $BRAF^{Mut}$, CPI use was higher in stage III (62%) than IV (52%) unlike in $BRAF^{wt}$ (52% in III vs. 90% in IV). BRAFi were used in 65% $BRAF^{Mut}$ (79% in IV vs. 34% in III). $BRAF^{Mut}$ and $NRAS^{Mut}$ received less adjuvant therapy than wild type (20-22% vs. 28-31%) but more LOTs ($BRAF^{Mut}$: 89% LOT 1, 37% LOT 2, 13% LOT 3, 5% LOT 4+). Comparing $BRAF^{Mut}$ (2011-14 vs. 2015-16), adjuvant use of vemurafenib (12% to 0) and cytokines (60% to 33%) fell but adjuvant use of dabrafenib/trametinib (4% to 9%), ipilimumab (14% to 35%), nivolumab (0 to 9%), ipilimumab/nivolumab (1% to 7%), and pembrolizumab (1% to 7%) increased. In LOT 1, vemurafenib (28% to 5%), ipilimumab (23% to 8%) and cytokine (7% to 3%) use declined. CPI use doubled in 2015-16 in LOT 1-2: ipilimumab/nivolumab (15%-12%), pembrolizumab (13%-21%), nivolumab (10%-21%). In LOT 1-2 use of dabrafenib/trametinib (to 43%-38%) and cobimetinib/vemurafenib (to 3%-10%) increased. Checkpoint inhibitors have replaced other advanced melanoma therapies providing more options to $BRAF^{Mut}$ melanoma patients.

Comparative screening of skin-derived NCSCs, melanocytes, and melanoma developmental programs reveals LPAR1 in MAPKi resistance

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Despite the high efficacy of BRAFi/MEKi in *BRAF*-MT melanomas, resistance arises in the majority of cases. Melanoma hijacks developmental pathways that drive aggressiveness, however gene signatures shared by melanocyte progenitor cells and melanoma remain poorly understood. Here, we define common dependencies in neural crest stem cells (NCSCs) and melanoma not present in melanocytes through computational transcriptome analyses and targeted siRNA screens against shared developmental genes. Secondary validation coupled with Ingenuity Pathway Analysis identified the LPAR1-RAPGEF5-RAP1A axis as the top pathway critical for stem cell maintenance and melanoma aggressiveness. Genome-wide gene expression data in the CCLE demonstrates LPAR1 correlates with the $MITF^{lo}/AXL^{hi}$ intrinsic MAPKi resistance signature. In agreement, LPAR1 is elevated in MAPKi-resistant PDX models, and in a subset of post-treatment tumor biopsies from patients who relapsed on MAPKi, relative to respective paired pre-treatment biopsies. Melanocytes and fibroblasts express low levels of LPAR1 and are not sensitive to LPAR1i. In contrast, genetic silencing of *LPAR1* and pharmacological inhibition of LPAR1 with HA130, an upstream LPAR1 inhibitor, triggers cell cycle arrest and anti-tumor activity in MAPKi-resistant cells *in vivo*. Mechanistically, genetic and pharmacologic targeting of LPAR1 down-regulates genes involved in PI3K/mTOR and Hippo/YAP signaling. Concurrent depletion of YAP and S6K significantly impairs melanoma

viability relative to knockdown of YAP or S6K alone, suggesting that Hippo/YAP and mTOR/S6K pathways are the main downstream effectors of LPAR1 signaling in melanoma. Our data identifies novel pathways responsible for the escape of *BRAF* mutant melanoma from MAPKi therapy and our hypothesis is that concurrent BRAFi/MEKi/LPAR1i may have therapeutic efficacy.

B cell signature predicts survival in melanoma and response to immune checkpoint blockade (ICB)

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Treatment with immune checkpoint blockade (ICB) has revolutionized melanoma therapy, and several biomarkers of response have been identified. These have mainly focused on mutational load and cytotoxic T cell markers, however there is a growing appreciation of B cells and other immune subsets in response to ICB. We recently conducted a neoadjuvant ICB trial in patients with high-risk resectable melanoma, and identified a B cell signature in responders to therapy using Nanostring DSP.

To gain further insight, we next performed transcriptomic profiling in longitudinal tumor samples from this cohort. This demonstrated enrichment of B cell markers in responders, with significantly higher expression of B-cell related genes such as MZB1, BTLA, and IGLL5 in responders (R) vs non-responders (NR) ($p<0.0001$ for all). We performed a more targeted immune gene assessment using MCP counter, demonstrating that a B cell lineage signature was predictive of response at baseline ($p=0.036$) and early on-treatment ($p=0.038$). Next, we applied this score to the TCGA and found this B cell lineage score was correlated with improved survival ($p<0.0001$), even after adjusting for cytotoxic T cell score ($p=0.004$ and $p=0.003$, respectively).

Based on these findings, we next examined tissue sections from longitudinal tumor samples in our ICB trial cohort and found that the B cells were organized in tertiary lymphoid structures (TLS) along with CD8 and CD4 T cells and CD21 follicular dendritic cells. Further, the density of TLS was higher in R vs NR at baseline and on-treatment ($p=0.078$ and $p=0.001$, respectively), and ratio of tumor area occupied by TLS was also higher in R vs NR ($p=0.037$ and 0.002, respectively).

Together, these results highlight the potential significance for B cell signatures as prognostic and predictive factors for response to ICB in melanoma.

TERT OFFSETS OXIDATIVE STRESS AND IS A VULNERABILITY IN NRAS MUTANT MELANOMA

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Targeting RAS is one of the greatest challenges in cancer therapy. Oncogenic mutations in *NRAS* are present in over 25% of melanomas and patients whose tumors harbor NRAS mutations have limited therapeutic options and poor prognosis. Thus far, there are no clinical agents available to effectively target NRAS or any other RAS oncogene. An alternative approach is to identify and target critical tumor vulnerabilities or non-oncogene addictions that are essential for tumor survival. We investigated the consequences of NRAS blockade in NRAS-mutant melanoma and show that decreased expression of the telomerase catalytic subunit, TERT, is a major consequence. TERT silencing or treatment of NRAS-mutant melanoma with the telomerase-dependent telomere uncapping agent 6-thio-2'-deoxy-guanosine (6-thio-dG), led to rapid cell death, along with evidence of both telomeric and non-telomeric DNA damage, increased ROS levels, and upregulation of a mitochondrial anti-oxidant adaptive response. Combining 6-thio-dG with the mitochondrial inhibitor Gamitrinib attenuated this adaptive response and more effectively suppressed NRAS-mutant melanoma. Our study uncovers a robust dependency of NRAS-mutant melanoma on TERT, and provides proof of principle for a new combination strategy to combat this class of tumors, which could be expanded to other tumor types.

Lipophilic bisphosphates in preclinical models of human melanoma

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Small G proteins (Ras, Rho, Rab) require prenylation to participate in the signalling networks that are often upregulated in malignant cells. Prenylation inhibition thus provides an opportunity to interfere with tumor growth and progression. Nitrogen containing bisphosphonates are potent inhibitors of prenylation but the hydrophilic/lipophilic nature of the compounds has major pharmacological consequences. Accordingly, we compare *in vitro* and *in vivo* effects of a hydrophilic (zoledronic acid) and a lipophilic bisphosphonate (BPH1222) on proliferation, migration, apoptosis induction, spheroid and tumour growth inhibition in human melanoma cell models. Eight human melanoma cell lines were selected that cover various mutational groups such as BRAF mutant, BRAF and PTEN mutant,

NRAS mutant and wild type lines. We measured the impact on short and long term proliferation, migration, apoptosis induction via SRB assay, clonogenic assay, videomicroscopy and western blot, respectively. Furthermore, 3D spheroid and *in vivo* subcutaneous tumour growth was also studied. In the majority of cell lines, BPH1222 outperformed zoledronic acid. Of note, in the NRAS mutant M24met cell line zoledronic acid induced higher apoptosis and inhibited proliferation significantly better in 2D experiments. Interestingly, two cell lines had higher motility after treatment with zoledronic acid while with BPH1222 this adverse effect was not observed. In 3D spheroid experiments all cell lines were more sensitive to lipophilic BPH1222. Finally, *in vivo* experiments with M24met showed significantly better growth inhibition by BPH1222, in contrast to *in vitro* results. Sensitivity to lipophilic and hydrophilic bisphosphonates varied among the cell lines irrespective of the mutational background. Our findings suggest that further preclinical assessment of lipophilic bisphosphonates is warranted as potential antitumor agents.

In silico functional analysis of GWAS hits suggests mechanisms of predisposition to melanoma and longer telomere length

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About 20 genomic loci have been associated to cutaneous melanoma through genome-wide association studies (GWAS), but the mechanisms by which these loci increase risk remain mostly unknown. Recent sequencing and epidemiological studies have suggested that there is a shared genetic link between melanoma predisposition and having longer telomeres, and therefore in this *in silico* study we set out to fine-map the common genetic variants associated to both phenotypes and potentially altered biological mechanisms.
To do this, we first identified the regions overlapping between updated versions of the melanoma meta-analysis published in Law MH *et al* (2015) *Nat Genet* **47**(9):987-995 and the telomere length GWAS in Codd V *et al* (2013) *Nat Genet* **45**(4):422-427. Then, we performed genotype imputation, calculation of linkage disequilibrium metrics and data filtering to determine a list of potential SNPs associated to both phenotypes. Subsequently, we carried out functional annotation by imputing chromatin states, identifying transcription factor binding sites (TFBS) disrupted or created by these SNPs, and cross-referencing with databases such as the Genotype-Tissue Expression project and the Hi-C/Virtual 4C data visualizing tool at Penn State University.
These strategies yielded a list of SNPs in the *TERC*, *GPR37/POT1* and *OBFC1* genomic regions associated to both melanoma and telomere length, and identified potential mechanisms of action through the annotation of active chromatin state and altered TFBS predictions. These functional hypotheses will be tested experimentally.

Toward minimal residual disease-directed therapy in melanoma

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Many patients with advanced BRAFV600E/K-mutant melanoma achieve dramatic responses to concurrent BRAF/MEK inhibition, yet retain minimal residual disease (MRD) which ultimately results in relapse.
To gain insights into the biology of MRD we applied single-cell RNA-sequencing to malignant cells isolated from BRAF-mutant patient-derived xenograft (PDX) melanoma cohorts exposed to concurrent BRAF/MEK inhibition.
We identified distinct drug-tolerant transcriptional states which co-occurred in varying combinations within MRDs from PDXs and biopsies of patients on treatment. One of these exhibited a quiescent Neural Crest Stem Cell (NCSC) transcriptional program largely driven by the nuclear receptor RXRG. An RXR antagonist mitigated accumulation of NCSCs in MRD, while increasing the proportion of the other drug-tolerant states, and yet delayed the development of resistance to concurrent BRAF/MEK inhibition.
This data identifies the NCSCs as key drivers of resistance and illustrate the therapeutic potential of MRD-directed therapy. They also highlight how gene regulatory network architecture reprogramming may be exploited therapeutically to limit cellular heterogeneity, a key driver of disease progression and therapy resistance.

FOXD3 Regulation of VISTA expression in melanoma

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Inhibitory and stimulatory immune checkpoint proteins modulate the immune response to cancer. FDA-approved inhibitors that target the inhibitory immune checkpoint proteins PD-1/PD-L1 and CTLA-4 have revolutionized the treatment of melanoma, improving response durability and extending patient survival. Unfortunately, only 20-40% of patients respond to therapy. Thus, consideration of additional immune checkpoint proteins as therapeutic targets is essential to expand the scope of immune checkpoint blockade. The discovery of the immune checkpoint protein VISTA provides an exciting new target for therapy. VISTA is an understudied immumodulatory protein that can act as a ligand or receptor to inhibit T cells; however, its precise role in modulating the anti-tumor immune response is poorly characterized. Furthermore, VISTA is primarily expressed on immune cells and few studies have investigated the expression and regulation of VISTA on tumor cells. We observe that VISTA is expressed on melanoma cells in patient samples and cell lines, which has implications for its role in modulating T cell responses. VISTA has previously been linked to processes of stem cell differentiation, and we show that the stemness factor FOXD3 negatively regulates VISTA expression at both the protein and transcript level. Importantly, a DNA-binding impaired mutant FOXD3 does not alter VISTA levels, suggesting a transcriptional mechanism of regulation. Published studies in the lab demonstrated that FOXD3 is upregulated in response to BRAF inhibition. We observe that in addition to upregulating FOXD3, BRAF inhibition also downregulates the expression of VISTA. These findings broaden our understanding of the effects of BRAF inhibitors on the immune profile of melanoma cells. Furthermore, we identify a surprising connection between a stem cell differentiation factor and immune evasion pathways in melanoma.

miR-155-5p overexpression is associated with NFE2L2 down-regulation in the liver of melanoma B16-bearing mice at pre-metastatic stage

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MicroRNAs as posttranscriptional regulators take part in various cancer-related processes such as cell proliferation and growth, angiogenesis, interactions with microenvironment, etc.
Here we discovered that miR-21-5p, miR-155-5p were up-regulated whereas miR-205-5p was down-regulated in melanoma as compared to melanocytic nevi. To further investigate the role of microRNA in melanoma progression and metastasis development, we used melanoma B16 mouse model where microRNA levels in tumors and metastasis-targeted organs were determined. Melanoma cells absence in melanoma metastasis target organs at pre-metastatic stage was confirmed by the lack of premelanosome protein mRNA. miR-205-5p, miR-21-5p, and miR-155-5p levels were increased in the liver of melanoma B16-bearing mice versus controls. TargetScan 7.0, miRWalk 2.0, miRTarBase v.4.5, and miRDB v.4.0 computational tools were used to identify potential target genes of these miRs. Transcription factor NFE2L2 was found to be a gene target for miR-155-5p. Its levels in the liver of B-16 murine melanoma were determined by real-time PCR. NFE2L2 mRNA levels were 3 times lower in pre-metastatic liver versus control. Exosomes released by cancer cells are involved in the formation of premetastatic niche and may modulate microRNA level alterations in distant organs. Altered microRNA levels in metastasis-target organs can correspond to release of paracrine regulators by stromal cells or immune cells migrated there. NFE2L2 transcription factor is involved in antioxidant response activation, it's decreased levels in liver of B16 melanoma-bearing mice at pre-metastatic stage might be a part of stromal organs functional reorganization due to cancer process progression.
The study was supported by the grant of Russian Science Foundation (project n 14-15-00074P).

Prediction of overall survival (OS) benefit from relapse-free survival (RFS) benefit of adjuvant nivolumab (nivo) in completely resected melanoma

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Using individual-patient data from 12 interferon-based randomized clinical trials (RCTs), RFS was shown to be a surrogate for OS in the adjuvant therapy of stage II-III melanoma (Suciu et al, 2018). Here, we apply a model to predict final OS based on interim RFS analysis from the RCT of adjuvant nivo vs ipilimumab (ipi).

We used published hazard ratios (HR) for RFS and OS from the same 12 RCTs as indicated above, as well as from 3 recent RCTs: ipi vs placebo (EORTC 18071), nivo vs ipi (CheckMate-238), and dabrafenib/trametinib (D/T) vs placebo (COMBI-AD). We quantified the trial-level surrogacy of RFS for OS through linear regression models and coefficients of determination (R^2) in a primary (using the 12 RCTs) and two sensitivity analyses (13 RCTs, adding the ipi vs placebo RCT; and 14 RCTs, also adding the D/T vs placebo RCT). We used these models to forecast HRs for OS using the HR for RFS (0.66) observed in the ongoing nivo vs ipi RCT. We also forecasted worst-case scenarios using the upper 95% confidence limit for the RFS HR from that RCT (0.81). We estimated surrogate threshold effects (STE), the minimum HR for RFS that predicts a HR for OS <1.

The forecasted HR for OS for the nivo vs ipi RCT ranged from 0.65 to 0.72 (worst case OS HR, 0.82 to 0.84), with 95% prediction limits (PL) entirely below 1.00. These analyses suggest that adjuvant nivo might prolong OS, as compared with adjuvant ipi. The extent to which post-relapse therapy will influence these predictions is uncertain.

Relationship between relapse-free survival (RFS) and distant-metastasis-free survival (DMFS) and their potential as surrogates for overall survival (OS) in high-risk resected melanoma

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Using individual-patient data (IPD) from interferon-based randomized clinical trials (RCTs), RFS was shown to be a surrogate for OS in the adjuvant therapy of stage II-III melanoma (Suciu et al, 2018). Here we used surrogacy methods for IPD to study the associations between RFS and DMFS in EORTC-18071 (ipilimumab vs placebo) and CheckMate-238 (nivolumab vs ipilimumab). In addition, we evaluated RFS and DMFS as potential surrogates for OS in EORTC-18071.

Having only 2 RCTs, we used the geographic location of centers to increase the number of units of analysis. We measured the individual-level association between surrogates and each final endpoint using Spearman's correlation coefficient (ρ), and the trial-level associations (between hazard ratios) for surrogates and final endpoints using R^2. For both ρ and R^2, values closer to 1 indicate stronger associations required for surrogate validation. The precision of these measures is quantified by their 95% confidence intervals (CIs).

Since 83% of RFS events were or became DMFS events, there were strong individual- and trial-level associations (R^2 0.93 for EORTC-18071 and 0.87 for CheckMate238). A strong individual-level association was also established between RFS, DMFS and OS for EORTC-18071. R^2 estimates were 0.59 and 0.69, respectively, suggesting at least a moderate association between treatment effects on RFS or DMFS and OS. However, 95% CIs were wide.

The results using EORTC-18071 OS data suggest that the surrogacy of RFS for OS found in the interferon-based era seems to hold true, so far, with checkpoint inhibitors (CPIs), and the results with both RCTs lend further support to the use of DMFS in this setting. Analysis with OS data for CheckMate 238 is warranted to confirm surrogacy of RFS and DMFS for OS in trials with CPIs.

Treatment sequencing and survival outcomes in *BRAF* mutation-positive (BRAFmut) metastatic melanoma (MM) patients (pts) treated with immunotherapy in routine clinical practices in the US

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Development and approval of checkpoint immunotherapy (CPI) regimens has improved progression-free and overall survival (OS) benefit in BRAFmut MM. We describe treatment patterns and clinical outcomes of CPI in routine clinical practice in the United States.

BRAFmut MM pts receiving front-line (1L) aPD-1 therapy (pembrolizumab [PEM], nivolumab [NIVO], or ipilimumab [IPI]+NIVO) between 01/01/2014 and 11/30/2017 were identified [Flatiron Health Data, New York, NY]. Patient status was defined as 1) deceased; 2) alive, continuing or completed 1L ≤30 days of data cutoff with no evidence of subsequent treatment (2L); or 3) alive with evidence of 2L.

Of 677 BRAFmut MM pts treated 1L, 192 received aPD-1 therapy: IPI+NIVO, 79 (41%); PEM, 75 (39%); or NIVO, 38 (20%). At data cutoff (01/31/2018), 37 pts (19%) had died. Among 155 pts still alive, 74 pts (39%) were continuing or completed 1L with no evidence of 2L therapy and 81 pts (42%) had received 2L therapy. Median OS for pts exposed to 1L aPD-1 therapy was not reached (19.8–NA) and varied by lactate dehydrogenase (LDH) at baseline (LDH high: 13.2 [9.89–20.3] months and LDH low-normal: not reached [22.74–NA] months). OS with 2L therapy was 13.0 (8.3–NA) months and was also significantly different in LDH normal (19.7 [14.3–NA] months) vs LDH high (7.6 [6.1–NA] months). The most common reasons for discontinuing 1L therapy included disease progression (46 pts [57%]) and treatment toxicity (16 pts [20%]). BRAF-targeted therapy was utilized as 2L in 57 pts (70%).

This real-world analysis demonstrated that single or combination aPD-1 therapy was associated with shorter OS in pts with high baseline LDH levels in both 1L or 2L, and that BRAF-targeted therapy was used as a 2L option in a significant proportion of pts following 1L aPD-1 therapy.

Epigenetic regulation of MITF and SOX10 in melanoma

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Several studies have shown that therapy-resistant melanoma cells are characterized by loss of the melanocyte lineage specific transcriptional program, in which MITF and one of its critical regulator SOX10 play key roles. Recently, we found a negative correlation between *MITF* promoter methylation and MITF expression, suggesting that MITF activity may be epigenetically regulated. In this study we used immunohistochemical staining of MITF and SOX10 on tumor tissue from 177 melanoma metastases. For further characterize the tumors we obtained mutational, gene expression and genome-wide DNA methylation data. Moreover, by using RNA sequencing and genome-wide DNA methylation data, we selected and phenotypically explored melanoma cell lines harboring *MITF* promoter methylation.

Overall, 17% of tumors had loss of MITF protein while 6% had concomitant loss of MITF and SOX10. Importantly, we found that MITF loss was associated with worst prognosis in the metastatic setting (*P*=0.02). These subsets displayed differentially deranged signaling pathways, including complete shutdown of the melanocyte transcriptional program in cases with simultaneous loss of MITF and SOX10. Further supporting our previous findings, tumors with MITF loss were more likely to harbor *MITF* promoter methylation (*P*<0.001). In cell lines harboring *MITF* promoter methylation, we uncovered two distinct groups primarily separated by SOX10 expression. DNA methylation data demonstrated that SOX10 down regulation was strongly correlated with *SOX10* promoter methylation, suggesting epigenetic regulation of the *SOX10* gene. Phenotypically, melanoma cells with concurrent *MITF* and *SOX10* promoter methylation had increased migratory and colony forming capacity and decreased response to MAPK inhibition. In conclusion, we demonstrate that melanoma cells and tumors harboring *MITF* promoter methylation are associated with poor patient survival and distinct phenotypic features.

Retrospective Analysis of Pyrexia in Patients (pts) Treated With Dabrafenib (D) and/or Trametinib (T) in a Real-World Setting

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Pyrexia (body temp >38.0°C) is a common adverse event (AE) with D and/or T that may lead to interruption and/or discontinuation of treatment (tx). Lack of recognition/early intervention may result in complications (rigors, dehydration, hypotension, or renal failure). Here we characterize pyrexia and its management (mgmt) in pts treated with D and/or T outside clinical trials. A global safety surveillance database was queried for reports of pyrexia in pts treated with D and/or T using pyrexia and related terms. As of Apr 30, 2018, 1886 pyrexia events were reported in 1581 pts (n=1189 with melanoma) treated with D+T. Mean age was 57y; 46% male/42% female/11% not reported. Most events occurred early after initiation (median, 41d [range 0-819d]). Recurrent episodes were reported in 189 (12%) pts (2 [n=125]; 3 [n=38]; >3 [n=26]); median time between pyrexia episodes was 23d. Chills (20%), nausea (9%), rash (9%), and fatigue (9%) were the most common pyrexia-associated AEs. AEs suggestive of complicated pyrexia (dehydration [2.8%], renal insufficiency [2.6%] or hypotension [2.1%]) were infrequent. Pyrexia outcome was known in 1299 of 2195 cases in pts treated with D and/or T; 90% had resolved/were improving. Dose interruption was the most successful intervention in the mgmt of pyrexia (95% of events resolved/improving). Prednisolone ± NSAIDs was used in 65 cases. Concomitant infection was reported in 11% of cases; nasopharyngitis was most common. To isolate cases of pyrexia likely related to tx (and not to infection), an analysis was performed on cases in which only pyrexia (without any other terms) was reported (n=357). Median onset was 25d and 89% of cases had resolved/improved (96% in pts with dose interruption). These results suggest tx interruption is effective for mgmt of pyrexia. Healthcare providers should be cautious to rule out infection.

Modulation of cJUN-EDN1 Axis by Notch Overcomes MAP Kinase Inhibitor Resistance in BRAF(V600E) Mutant Melanoma Cells

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Notch signaling has been implicated in melanoma tumor initiation, progression and metastasis. However, preclinical and clinical studies targeting Notch signaling for treatment of melanoma have produced conflicting results. The role of Notch signaling in resistance of melanoma cells to Mitogen Activated Protein Kinase (MAPK)-targeted therapy has not been well established. In this study, we found that NOTCH1 is downregulated in melanoma cells with intrinsic and acquired resistance to MAPK inhibition (MAPKi). We show that overexpression of Notch Intracellular Domain (NICD), the active form of Notch, induced cell death in the NOTCH1-low, MAPKi-resistant cells, but not in the NOTCH1-high, MAPKi-sensitive melanoma cells. Whole transcriptome analyses identified differential regulation of EDN1 by Notch signaling in MAPKi-sensitive and -resistant cells, i.e., downregulation of EDN1 in MAPKi-resistant cells and upregulation of EDN1 in MAPKi-sensitive cells. Knockdown of EDN1 partially mimics NICD overexpression in MAPKi- resistant cells. Our data show that NICD activates apoptosis in the resistant cells, in part, by downregulating EDN1 through downregulation of c-JUN. These findings suggest that MAPKi-resistance is mechanistically linked to downregulation of Notch signaling and that reactivation of Notch signaling is sufficient to overcome the resistance to killing independent of MAPK inhibition. We propose that in the context of MAPKi resistance, melanoma cells downregulate Notch signaling in order to upregulate EDN1 as a survival pathway. Understanding the mechanisms of action of Notch in drug resistance will have an impact on the design of better therapeutic strategies for melanoma.

Molecular profiling of female genital and anorectal melanoma in a supra-regional melanoma centre

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Targeted immunotherapy has contributed to improved outcomes in metastatic cutaneous melanoma; however mucosal melanoma has a distinct genetic profile and is associated with a poorer prognosis. The aim of our study was to assess the pattern of molecular alterations among patients referred to our centre and to determine the value in separating vulval, vaginal and anal melanoma.
We identified patients with a histologically confirmed diagnosis of vulval, vaginal and anal melanoma. A retrospective review of mutational analyses was undertaken for BRAF, KIT and NRAS.
27 patients were identified with vaginal melanoma (avg age 72, 49-95), 34 with vulvar melanoma (avg age 76, 44-93) and 31 with anal melanoma (avg age 71, 37-96, 38.7% male, 61.3% female), referred from 33 separate hospital sites in the UK between 2012 and 2017. Of those with vaginal melanoma, 0% had KIT mutation (0/12), 0% BRAF mutation (0/23) and 0% NRAS mutation (0/1). For vulval melanoma, 17% had KIT mutation (3/18), 2.9% BRAF mutation (1/34) and 0% NRAS mutation (0/3). For anal melanoma, 0% had a KIT mutation (0/15), 6.6% BRAF mutation (2/30) and 0% NRAS (0/3).
Our data demonstrate a low level of BRAF and NRAS mutations in our cohort of female genital and anal melanoma, supporting the notion that these subtypes are genetically distinct from cutaneous melanoma. Overall KIT mutation rates in combined literature were 24.26% for vulvar melanoma, 5.13% for vaginal and 19.67% anal. Our data concord with the value of KIT testing in vulval melanoma but not vaginal melanoma and justify separating these subtypes.
Current molecular targeted immunotherapy for cutaneous melanoma are not useful for the majority of these patients. This suggests that new mutations must be identified in this subgroup. This may be possible through entry into future initiatives such as the 100,000 genome project using next generation sequencing.

Targeting the PRMT5-MDM4 axis in melanoma

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PRMT5 by methylating arginine residues on histones and non-histone proteins regulates many cellular processes including gene expression, cell signalling and pre-RNA processing. PRMT5 activity is regulated by MEP50, which is phosphorylated and activated by CDK4/Cyclin D1. In most melanomas, PRMT5 and MEP50 expression is elevated and CDK4 signalling is hyper-activated due to loss of the tumour suppressor p16^{INK4A} or activation of the MAPK/ERK pathway. Thus, CDK4 driven MEP50/PRMT5 activity may play a significant role in this disease. In studies addressing acquired resistance to CDK4 inhibition in melanoma, loss of inhibition of PRMT5 activity and activation of CDK2 were identified as conferring resistance. Further studies revealed that in drug sensitive cells inhibition of CDK4 or PRMT5 decreased expression of the oncoprotein MDM4 by altering the splicing of MDM4 pre-mRNA. Loss of MDM4 protein expression led to p53 activation, increased p21 expression and inhibition of CDK2. In p53 mutant cell lines loss of MDM4 expression was also very effective at inhibiting cell proliferation, indicating p53-independent effects of MDM4 in these cells. GSK3326595 (PRMT5i) enhanced the response to palbociclib and MAPK/ERK pathway inhibitors leading to robust inhibition of melanoma cell proliferation and tumour growth. Our studies have identified that PRMT5 inhibitors potently decrease MDM4 expression, overcome CDK4 inhibitor resistance and enhance the efficacy of CDK4 and MAPK-targeted therapies.

Results From an Open-Label, Phase 2a Study of Dabrafenib Plus Trametinib (D+T) in Asian Patients (pts) With Advanced *BRAF* V600–Mutant Cutaneous Melanoma

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D+T has been extensively studied in phase 2/3 trials in pts with unresectable or metastatic cutaneous melanoma. However, those trials enrolled mainly non-Asian pts and thus provided limited direct evidence of the efficacy and safety in Asian pts. This phase 2a study enrolled adult pts of East Asian descent with stage IIIC/IV *BRAF* V600–mutant cutaneous melanoma (NCT02083354). Prior treatment (tx) was allowed (except BRAF/MEK inhibitors). Pts received the approved dose of D 150 mg BID and T 2 mg QD. The primary endpoint was objective response rate (ORR). Secondary endpoints were duration of response (DOR), progression-free survival (PFS), overall survival (OS), pharmacokinetics, and safety. At the data cutoff (median follow-up, 8.25 mo), 77 pts were enrolled; tx was ongoing in 36 (47%). The median age was 52 y; 55% were female, 58% had M1C disease, 65% had ECOG PS of 1, 32% had elevated LDH, and 84% had ≥1 prior therapy. Median duration of exposure was 7.0 mo with D and 6.5 mo with T. The ORR was 61% (47/77 pts); 4 (5%) and 43 (56%) pts achieved complete and partial responses, respectively. Median DOR and PFS were 11.3 mo and 7.9 mo. Median OS was not reached (23 pts died). Mean C_{max} for D and T was 3560 ng/mL and 11.5 ng/mL (day 1) and 2680 ng/mL and 27.1 ng/mL (day 15). The most common adverse event (AE) of any grade was pyrexia (56%). Grade ≥3 AEs occurred in 29 pts (38%). The most common grade ≥3 AEs of special interest were pyrexia and neutropenia (6 pts [8%] each). AEs led to permanent discontinuation in 3 pts (4%). Median PFS was shorter than previously reported results in non-Asian pts, and was potentially influenced by allowance of prior tx and higher baseline ECOG PS in this study. Overall, these results support the efficacy and tolerability of D+T in East Asian pts with advanced *BRAF* V600–mutant melanoma.

Inhibition of melanoma cell derived prostaglandin E2 amplifies T-cell receptor signalling and shows clinical efficacy in combination with anti-PD1 therapy

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Background
It is well known that melanoma cells actively suppress immune cell function and activity via the expression of suppressive ligands of checkpoint inhibitors like PD-L1. Here, we investigated whether melanoma cells actively secrete factors that impair T-cell receptor signaling in T-cells.
Methods
With the help of a luciferase based T-cell reporter cell line we analyzed the effects of melanoma cells and secreted factors on T-cell receptor signaling. By ELISA and mass spectrometry we identified and quantified prostaglandin E2 (PGE2). Aspirin and celecoxib were used as cyclooxygenase inhibitors to treat melanoma cell lines and short-term cultured patient derived metastatic melanoma cells to prevent PGE2 synthesis.
Results
We could show that melanoma cell supernatants actively suppressed IL2-promoter activity after stimulation of the TCR. We further identified the hormone-like lipid PGE2 as a main mediator of this effect. PGE2 was secreted in effective concentrations from several melanoma cell lines and short-term cultured patient derived melanoma cells. COX-2 inhibition using either aspirin or celecoxib not only abolished PGE2 secretion but also significantly diminished the suppressive effect of melanoma conditioned medium on T-cells. In line with the work of Zelenay et al. two patients with moderate progressive disease under anti-PD-1 therapy shifted into long lasting regressions after the addition of aspirin as shown by PET/CT scanning. In addition, aspirin intake tended to be associated with a better response rate and better PFS in melanoma patients receiving anti-PD-1 based immunotherapy.
Conclusion
We therefore hypothesize, that human metastatic melanomas use a cyclooxygenase dependent evasion of immunity that could be tackled by the concerted action of COX and checkpoint inhibitors.

RNA binding kinase UHMK1 regulates therapy induced metabolic adaptation in BRAF[V600] melanoma

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Despite the success of therapies targeting oncogenes in cancer, clinical outcomes appear to be limited by a drug adaptation and tolerance phase. Metabolic adaptation in response to MAPK pathway inhibition is well established in melanoma, and several metabolic vulnerabilities including glycolysis, oxidative phosphorylation and glutaminolysis have been linked with therapeutic response, adaptation and resistance. To examine how therapy reprograms metabolism we performed a genome-wide RNAi screen in melanoma cells following BRAF inhibition in the therapeutic adaptation phase prior to acquired resistance. This approach uncovered mRNA transport and translation, including RNA binding kinase UHMK1, as a critical regulator of metabolic responses to BRAF inhibition. Depletion of UHMK1 enhanced BRAF inhibitor sensitivity, synergistically suppressing glycolysis, proliferation, and viability, whilst analysis of mitochondrial metabolism revealed reduced spare respiratory capacity, ATP production and glutamine dependency. Together this data identifies a multifaceted role for UHMK1 in metabolic responses to BRAF inhibition. Mechanistically, polysome profiling and *de novo* protein synthesis assays revealed selective translation of mRNA encoding metabolic enzymes in cells adapting to BRAF inhibition, and critically, we show this is UHMK1 dependent. Moreover, we demonstrate UHMK1 interacts with mRNA encoding these enzymes, and regulates their nuclear-cytoplasmic transport. Our data suggests UHMK1 regulates therapy-induced metabolic adaptation by controlling the abundance of metabolic enzymes through the export and translation of the mRNA that encode them. We propose this pathway is an attractive therapeutic target to improve efficacy of MAPK pathway inhibitors by targeting the process of adaptation itself, rather than the outcome, as a next generation combination therapy.

Whole-exome sequencing of acquired nevi identifies mechanisms for development and maintenance of benign neoplasms

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The melanoma transformation rate of each nevus is rare despite the detection of oncogenic *BRAF* or *NRAS* mutations in 100% of nevi. Acquired melanocytic nevi (AMN) do however mimic melanoma and ~30% of all melanomas arise within pre-existing nevi. Using whole-exome sequencing of 30 matched nevi, adjacent normal skin, and saliva we sought to identify the underlying genetic mechanisms for nevus development. All nevi were clinically, dermoscopically, and histopathologically documented. In addition to identifying somatic mutations, we found mutational signatures relating to ultra-violet radiation (UVR) mirroring those found in cutaneous melanoma. In nevi we frequently observed the presence of the UVR mutation signature compared to adjacent normal skin (97% vs 10% respectively). In copy number aberration (CNA) analysis, in nevi with copy number loss of tumor suppressor genes (TSG), these were balanced by loss of potent oncogenes. Moreover, reticular and non-specific patterned nevi revealed an increased (p<0.0001) number of CNA as compared with globular nevi. The mutation signature data generated in this study confirms that UVR strongly contributes to nevogenesis. Copy number changes reflect at a genomic level the dermoscopic differences of AMN. Lastly, we propose that the balanced loss of TSGs and oncogenes is a protective mechanism of AMN.

Proteomic analysis of serum proteins detected in malignant melanoma and their inter-cellular crosstalk in the tumour microenvironment

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Malignant melanoma (MM) is a life-threatening predominantly cutaneous cancer. MM arises from the melanocytes, the pigment-producing cells. MM, as well as the other tumours, represents a complicated network of malignant cells and various nonmalignant cell types, namely cancer-associated fibroblasts, leukocytes, pericytes or endothelial cells. Collectively, all these cell types together with extracellular matrix form so called tumour microenvironment (TM). The TM is able to influence the biological aspects of cancer cells, especially the growth and progression.
Progression of MM and its aggressive spreading are associated with systemic response e.g. changes of different serum proteins. Here we exploit a comparative serological analysis of serum samples from MM patients using LUMINEX approach. In parallel, we detected a panel of the selected proteins in primary MM tumours. This comparative analysis showed a specific pattern of serum proteins (HGF, VEGFA, GCSF, IL6, IL1RA, IFNα, IL8) in distinct clinical stages of MM. We also demonstrate in vitro cocultivation of MM cells with normal or cancer-associated fibroblasts. ELISA test for interleukins 6 and 8 respectively proved the tumour-supporting effect of cancer-associated fibroblasts. These experiments provided deeper insight into biological properties of MM cells and demonstrated the interaction of this tumour with cells forming its TM.

High naevus count, *MC1R* RHC alleles and melanoma risk

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A high number of acquired melanocytic naevi is the strongest known phenotypic risk factor for cutaneous melanoma; individuals with the highest numbers of naevi can have a 5 to 10-fold increase in melanoma risk. The red hair colour (RHC) phenotype, produced by *MC1R* R-variant alleles and consisting of red hair, freckling and pale skin that burns easily and does not tan well, also predisposes to melanoma. Melanoma risk is elevated approximately 4-fold in individuals with the RHC phenotype, caused by highly penetrant variants in *MC1R*. However, the study of both phenotypic risk factors in combination with *MC1R* genotype has not been directly reported. To address this question we have used 1267 participants from South-East Queensland, Australia, recruited as part of the Brisbane Naevus Morphology Study (BNMS), an epidemiological case-control study of melanoma and naevi. Participants underwent full body imaging and phenotypic data including pigmentation characteristics and number of naevi ≥5mm were collated. *MC1R* genotype was determined using genomic DNA prepared from a saliva sample. Linear and logistic regressions were used to assess the importance of naevus count, red hair, and *MC1R* genotype as risk factors for melanoma. Compared to individuals with dark brown hair and 0-4 naevi, individuals with red hair and 20+ naevi had a greater than 10 fold risk of melanoma. Individuals with *MC1R* R/R genotype and 20+ TNC≥5mm had a 25-fold increase in risk compared to WT/WT individuals with 0-4 naevi. The highest risk group, Australian men with *MC1R* R/R genotype and 20+ moles, had an absolute risk of melanoma to age 75 of 23.3%, compared to 0.8% for men with WT/WT genotype and 0-4 naevi. Thus patients with many large naevi and RHC phenotype, particularly those with an *MC1R* R/R genotype, have an unusually high risk of melanoma and should undergo regular skin checks.

Phenotypic and genotypic analysis of amelanotic/hypomelanotic melanoma patients from an Australian case-control study

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Amelanotic/hypomelanotic melanoma (AHM) is a subtype where the tumour exhibits little or nomelanin pigmentation, and is commonly mistaken for other cutaneous conditions. It is associated with poorer diagnostic accuracy and a more advanced stage of disease at diagnosis. We have examined the genotype and phenotype profile of patients with an AHM to assist in profiling high risk patients. The Brisbane Naevus Morphology Study (BNMS) is an epidemiological case-control study of naevus and melanoma risk participants recruited from South-East Queensland. Histopathology reports and phenotype data were collected for 436 patients. Genotyping information allowed testing of variants in known pigmentation and melanoma genes including *MC1R*, *TYR*, *HERC2*/*OCA2*, *IRF4*, *PLA2G6* and *MITF*. We identified 389 patients with a pigmented melanoma and 47 patients with at least one AHM. The average age of patients with AHM was significantly higher than patients with pigmented melanomas (63.3 vs 54.6 years; P<0.001). *MC1R* R/R genotype was at an almost 3 fold higher level in our AHM population (31.1%) in comparison to the pigmented melanoma group (11%) (p<0.001; OR 26.4 vs 5.9). Allele associations identified as significant in AHMs vs pigmented melanomas include *TYR* R402Q 42.1% vs 32.1% (p=0.06), *MTAP* rs4636294*G (53.4% vs 41.7%; p=0.02) and *PLA2G6* rs11570734*G (23.9% vs 36.6%; p=0.02). *MTAP* rs4636294*A/A (OR of 0.8 vs 2.0) and *PLA2G6* rs11570734*A/A (OR of 3.7 vs 1.3) naevus/melanoma genotypes were significantly associated with AHM but surprisingly with the *MTAP* protective allele being greater in AHM patients. Knowledge of phenotypic and genotypic associations of AHM melanoma can help predict risk of this difficult to diagnose melanoma, which may assist clinical management and patient skin self-examination

Nab-paclitaxel and carboplatin combined with anti-angiogenetic drug in advanced melanoma patients progressing on previous regimen(s).

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Objective: This study was to investigate the efficacy and safety of nab-paclitaxel(nab-p) and carboplatin combined with anti-angiogenetic drug in unresectable metastatic melanoma patients(pts) progressing on previous regimen(s).
Methods: Previously treated patients with unresectable stage IIIC or IV melanoma were enrolled. The treatment regimen consisted of a 28-day cycle in which patients received nab-paclitaxel (260 mg/m^2) and carboplatin(AUC=5) through intravenous (IV) infusion on day 1 and bevacizumab, 5 mg/kg IV every 2 weeks or endostatin 15mg days 1-14. The 28-day cycle was repeated until there was unacceptable toxicity or disease progression. The primary endpoint was the progression-free survival (PFS), while the secondary end points were objective overall response（ORR）, overall survival (OS) and safety profile.
Results: Eighty-seven patients were enrolled from 2015-2017 with median age 57y, including 55% males, 13% brain mets, 92% stage IV, 22% LDH>2.5×ULN. Of all the pts, 26% failed from 1st line therapy, while 43% and 31% had 2nd line or more than 3rd line regimens previously. There were 15 partial responses and no complete responses yielding an overall ORR of 17%. The median PFS was 3.0 months and the median OS was 9.2 months with a median duration follow-up of 22.6 months. There were no significant differences in PFS or ORR between subgroups of bevacizumab and endostatin. Common adverse events associated with this regimen were fatigue, alopecia, neutropenia, gastrointestinal disorders, and peripheral neuropathy. Thirty-three percent of the pts were treated with dose reduction，10% discontinued therapy due to toxicities.

Conclusion: This triplet regimen demonstrate rapid antitumor effects even in those who progressed on multiple prior therapies with tolerable adverse effects.

Treatment-free survival (TFS), a novel outcome applied to immuno-oncology (IO) agents in advanced melanoma (AM)

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Patients (pts) discontinuing IO agents may experience periods of disease control without needing subsequent systemic anticancer therapy (Rx). We propose TFS to characterize antitumor activity and toxicity of this period. Data were pooled from the CheckMate 067/069 trials of ipilimumab (I) and nivolumab (N) alone or in combination (N+I) for AM (407 N+I, 313 N, 357 I). I was given for 4 doses, and N until progression/intolerability. We defined TFS as the area between Kaplan-Meier (KM) curves for 2 time to event endpoints: (A) duration of protocol Rx (randomization until Rx cessation); (B) subsequent Rx-free survival (randomization until initiation of subsequent Rx or death). TFS with toxicity was alternatively characterized as TFS with treatment-related grade 3/4 AEs, or with grade 2-4 AEs. Area under each KM curve was estimated by the 36mo restricted mean time to event. Area under the overall survival (OS) curve was partitioned as time on protocol Rx (mean A), TFS (mean B-A), and post-subsequent Rx time (mean OS-B), and summarized as % of the 36mo period.

At 36mo, 58% N+I, 52% N, 36% I pts were alive. Few remained on protocol Rx (11% N+I, 17% N, 0% I) and many were surviving free of subsequent Rx (47% N+I, 37% N, 15% I). Mean OS was longer for N+I (25.7mo) and N (24.9mo) than I (21.4mo). Mean TFS was 31% of time for N+I (11.1mo) vs 13% for N (4.6mo), as N+I had shorter mean protocol Rx duration and longer time until subsequent Rx, and was 24% for I (8.7mo). TFS with toxicity represented a small proportion of TFS for all treatments.

Defining TFS by the area between KM curves for time to protocol Rx cessation and time to subsequent Rx or death showed AM pts receiving N+I spent more time free of Rx and without grade 3/4 AEs compared to N or I, which characterizes patient well-being, while treatment free.

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Initial systemic therapy impacts incidence of CNS metastases (mets) in patients (pts) with metastatic melanoma (MM): real-world evidence

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CNS mets are a major source of morbidity/mortality in MM. Immune checkpoint inhibitors (IT) and targeted therapies (TT) have shown CNS activity but their impact on incidence of CNS mets is not known. Data recorded between January 2011–March 2018 in Flatiron Health Data, NY were queried. CNS mets were identified by ICD-9/10 codes 198.3, C79.31/32. Based on metastatic diagnosis date (dx_{met}) 2 pt cohorts were created; 2011–2014 (n=1882) and 2015–2018 (n=2125), reflecting different eras in access to IT and TT. Treatment groups were based on therapy received ≤90 days after dx_{met} (any IT > no IT, any TT > no IT/TT, any chemotherapy/other [CT] > no systemic therapy). Of 4007 pts with MM with ≥1 visit ≤90 days after dx_{met}, 760 (19%) had CNS mets, 68 at dx_{met}, and 692 experienced interval development of CNS mets. Pts with CNS mets were younger (<65 y 60% vs 48%) and more frequently *BRAF* mutation-positive (47% vs 34%), but the prevalence of elevated lactate dehydrogenase levels was similar (both 16%). Comparing 2011–2014 to 2015–2018, more patients in the later cohort received systemic therapy (58% vs 40%), fewer pts received CT (2% vs 8%), and more pts received IT (47% vs 22%), while TT remained stable (12% vs 11%). Among pts treated with IT, aCTLA-4 use declined across cohorts (21.2% vs 6.9%), while aPD(L)-1 and aCTLA-4+PD(L)-1 use increased, 0.3% vs 25.0% and 0.1% vs 14.4%, respectively. In the 2011–2014 cohort, the cumulative incidence of CNS mets was similar across treatments (Gray's test *p*=0.6580; 3-y rates 21–23%) while a significant difference was noted in 2015–2018 cohort, (Gray's test *p*=0.0008; 3-y rates 19–34%). Notably, initial systemic treatments appear to affect outcomes in association with increasing rates of IT utilization. In conclusion, modern treatments, particularly IT, may reduce the incidence CNS mets in pts with MM.

Impact on overall survival of concomitant radiotherapy in patients with melanoma brain metastases using the propensity score matching study within MelBase, a French multicentric prospective cohort

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Background:
Melanoma brain metastases (MBM) are historically associated with poor prognosis. Radiation therapy is associated with a high local control rate. Recently, the development of targeted therapy and immunotherapy has improved overall survival and intra-cranial response rate, but about 50% of patients failed to respond to these novel therapeutics. The objective of this study was to assess the impact of concomitant radiotherapy (cRT) on overall survival in a large multicentric real-life prospective cohort of MBM patients treated with immunotherapy or targeted therapy.
Patients and methods:
Clinical data from 262 MBM patients were collected via MelBase, a French multicentric biobank prospectively enrolling unresectable stage III or IV melanoma. Two groups were defined: patients receiving (cRT group) or not (no-cRT group) cRT. Primary end-point was overall survival (OS). Propensity score weighting was used to correct for indication bias. The following variables were considered: sex, age, treatment line, BRAF mutation status, ECOG status, LDH level, liver metastases, symptomatic MBM, corticosteroids and number of metastases sites.
Results:
Among the 262 patients, 93 (35%) received cRT (cRT group). The patients were treated with immunotherapy in 69% and 60% and targeted therapy in 31% and 40% of the cRT and no-cRT group respectively. With a median follow-up of 6.9 months, median OS was 16.8 (IC 95%: 11.8 – 27.9) and 6.9 months (IC 95%: 5.4 – 9.4) in the cRT and no-cRT group respectively. After propensity score matching, concomitant RT was associated with higher OS (HR=0.6; p=0.008).
Conclusion:
This study demonstrates that concomitant radiotherapy is associated with a significant decrease of 40% in the risk of death in the MBM patients treated with systemic therapy.

Machine learning applications to predict prognosis in the Leeds Melanoma Cohort

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Machine learning methods have shown great potential for identification of prognostic biomarkers. Here, we tested whether machine learning can predict outcome in one of the largest population-based cohorts of primary melanoma, the Leeds Melanoma Cohort, using whole-genome tumour transcriptomes. The tumour samples (n=525) were divided into training (70%) and test sets (30%). Random forest (RF) and Support Vector Machine (SVM) methods were applied to the training set to develop classification models for predicting outcome, which was defined as whether or not the patient died from their melanoma within 6 years of diagnosis. The model performance was assessed in the test set using kappa index (k). We tested the hypothesis that adding clinical features (AJCC stage, age, sex and tumour site) into the transcriptome-based model would further improve the prediction performance.

The training set was imbalanced (69% survivors and 31% non-survivors), and RF and SVM classification models were trained in conjunction with correcting this imbalance. RF and SVM showed a good agreement between their predictions in the test set (Cramer's V- 0.73). In both methods, undersampling the majority group to achieve a balanced design provided the best performance (RF k=0.41, SVM k=0.31) compared to using imbalanced data (RF k=0.23, SVM k=0.27). Integrating clinical features into the RF model had similar performance (RF k=0.37) in comparison to the model generated using clinical features alone (RF k=0.38). Gene selection identified a combination of 50 genes, which, when combined with clinical features in RF, had the best performance in the test set (k=0.49). This model is currently being validated in an independent cohort of primary melanomas from Lund, Sweden. In conclusion, we have developed a machine learning model that predicts prognosis in primary melanoma. In future this model may be useful in clinical settings.

Consensus clustering of early stage melanoma reveals transcriptomic signatures with prognostic value.

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Previously published transcriptomic signatures of cutaneous melanoma have been generated predominantly from metastatic tumours. We have evaluated the prognostic significance of these signatures in a primary tumour transcriptomic dataset (N=702) sourced from the

Leeds Melanoma Cohort (LMC). Although they predicted outcome when analysed as a whole ($P=10^{-8}$ to 10^{-4}) when stratified by AJCC stage, these signatures did not predict outcome in LMC stage I tumours ($P=0.3$ to 0.7) and showed significant interaction with AJCC stage ($P=0.04$).

To generate a new signature, we re-analysed the LMC dataset by Partitioning Around Medoids in an unsupervised consensus clustering framework. This revealed six novel LMC classes with differentially expressed genes (DEG) which significantly predicted outcome in the whole LMC dataset ($P=10^{-7}$) and within disease stages, including stage I ($P=0.01$). Network enrichment analysis of DEG revealed distinctive biological characteristics of LMC classes and identified central 'hub' genes. In stage I tumours, one LMC class exhibited the worst outcome despite a convincing immune cell component; biological characterisation of this class displayed evidence for activation of epithelial to mesenchymal transition, upregulation of the 'hub' proto-oncogene *JUN* and transcription factor *NFKB1*. Next generation sequencing of a subset of LMC primary tumours (N=266) established *JUN* as commonly associated with somatic amplifications in this LMC class.

We present a novel melanoma transcriptomic signature, which, unlike previously reported signatures, predicts outcome in stage I disease. This novel melanoma signature delineates a subset of early stage tumours, which, despite histological characteristics indicative of good prognosis, are characterised by poor outcome. The LMC 6-class signature suggests the possibility of stratified clinical management of stage I patients.

Loss of tumor antigens drives cross-resistance to CD8^{+} T cells in MAPK-inhibitor-resistant melanoma cells

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Numerous clinical trials combining BRAF inhibitors (BRAFi) and immunotherapy are currently ongoing in melanoma patients to further enhance the profound but frequently transient clinical responses of BRAFi-based targeted therapy. To support the development of a rationale for the optimal treatment protocol, we evaluated the recognition of BRAFi-exposed melanoma cells by autologous tumor infiltrating CD8^{+} T cells *in vitro*. In different patient models, we observed the evolution of melanoma resistance to the pre-existing tumor-specific T cell repertoire during prolonged BRAFi exposure, as mono-treatment or combined with MEKi. Those long-term BRAFi-treated melanoma cells showed a non-proliferative senescence-like phenotype and were less sensitive to four out of five CD8^{+} T cell clones, present in the pre-existing TIL repertoire, of which three recognized shared antigens (Tyrosinase, Melan-A and CSPG4) and one being neoantigen specific. Notably, in all cases the impaired T cell activation was due to a time-dependent downregulation of their respective target antigens. Moreover, re-expression of the lost antigens in drug-resistant tumor cells restored T cell activation. In summary, MAPKi strongly alter the tumor antigen expression profile over time. Consequently, loss of tumor antigens in acquired drug-resistant tumor drives cross-resistance to T cells, suggesting that simultaneous treatment with MAPKi and immunotherapy could be most effective for tumor elimination.

Development of a 40-CpG Methylation-based Diagnostic Assay for Melanoma

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Because the histopathological diagnosis of cutaneous melanoma can be challenging for pathologists, we sought agnostically to determine if DNA methylation differences could discriminate melanoma from nevi. Illumina Infinium HumanMethylation450 BeadChip (450K) array analysis was performed on 89 primary invasive melanomas and 73 nevi from formalin-fixed paraffin-embedded specimens with diagnostic consensus by three dermatopathologist reviewers. The melanomas had median Breslow thickness of 1.85 mm (range 0.37-17.00 mm), were balanced for 7th Edition American Joint Committee on Cancer tumor stages, and included common and less common histopathological subtypes. The set of nevi included intradermal, common acquired, congenital pattern, dysplastic, Spitz, and other less common subtypes. Melanomas and nevi were randomly divided into training (60 melanomas, 48 nevi) and validation (29 melanomas, 25 nevi) sets. Predictive modeling using ElasticNet applied to the training set identified a 40-CpG melanoma classifier associated with 38 genes. The probe set utilized in the modelling was restricted to probes with β interquartile range (IQR) > 0.2 β, with gene annotation, and present on both the Illumina 450K and 850K arrays. Independent validation of the 40-CpG classifier in the validation set found high diagnostic accuracy (Area Under the Receiver Operating Characteristic curve = 0.996, sensitivity = 96.7%, specificity = 100.0%). Further, DAVID gene ontology analysis indicated that the 40-CpG melanoma classifier was enriched in homeobox genes, other transcriptional regulatory genes, and genes involved in neurological processes. In summary, the 40-CpG methylation-based diagnostic classifier shows promise as a tool for molecular diagnosis of melanoma, and the individual CpGs in the classifier provide potential new avenues for investigating melanoma's biological underpinnings.

Identification of mechanisms of resistance to BRAF/MEK inhibition through a lentiviral insertional mutagenesis screen

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Targeted therapies, inhibiting BRAF and MEK, have revolutionised the treatment of BRAF mutant melanoma. Duration of response is, however, limited by drug resistance, and a more complete understanding of mechanisms of drug resistance is needed to develop more effective therapies.

Here we applied a forward genetic approach of lentiviral-vector based insertional mutagenesis to screen for novel genes and pathways involved in resistance to BRAF/MEK inhibition. We employed lentiviral vectors designed to integrate semi-randomly in the genome and to activate nearby genes in order to look for culprits of resistance to the drug combination. We transduced a BRAF^{V600E} short term melanoma primary cultures with the lentiviral vector and then treated them with a BRAF/MEK inhibitor combination until the emergence of resistant colonies. Integration sites were then retrieved from resistant cells and controls and Gaussian Kernel Convolution-based statistical approaches were applied to identify significantly enriched clusters of integrations that flanked drug resistance genes.

We identified 9 candidate resistance genes to the BRAF/MEKi combination. These genes were enriched for activators of RAS and ERK, including candidates such as *SOS1* and *TRAF3*. We successfully validated our top hit, *SOX6*, showing that overexpression induced resistance to BRAF/MEK . We are currently investigating the molecular mechanism of drug resistance.

Overall we have identified a collection of candidate resistance genes to BRAF/MEKi, which may guide drug development or point to other rational drug combinations to target treatment resistance.

Detection of Novel Mutations in *TTC28* and *SF3B1* using Targeted Next Generation Sequencing (NGS) Panels for Uveal Melanoma.

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Integrating somatic copy number variations (CNV) and mutational status is important when determining prognosis for uveal melanoma (UM) patients. Using two custom designed NGS panels we examined CNVs in chrom. 1, 3, 6 & 8 and mutations in *GNAQ*, *GNA11*, *BAP1*, *SF3B1*, *EIF1AX*, which are associated with UM development and metastasis. We also examined the following genes for which infrequent mutations have previously been reported in UM; *TTC28*, *CSMD1*, *KTN1*, *DLK2*, *TP53BP1*, *SRSF2*, *CYSTLR2*.

All UM samples were from consenting patients (8 males/6 females; median age at diagnosis, 60yrs) and the study was ethically approved. Primary treatment was enucleation in 12 and local resection in 2. DNA was extracted from 14 snap frozen UM samples; all 14 had previous CNV data and 8 had mutation data from TCGA analyses. Target enrichment was performed for all tumours and two reference standards using Agilent SureSelect (SS) and Illumina TruSeq Custom Amplicon (TSCA), according to manufacturer's instructions. Variants were identified and annotated with SnpEff version 3.2a and Integrative Genomics Viewer.

In addition to detecting mutations in *GNAQ (29%)*, *GNA11 (50%)*, *BAP1 (50%)*, *SF3B1 (21%)*, *EIF1AX (7%) and CYSLTR2 (7%)* both panels detected a novel missense mutation of unknown significance in p.I1296V *TTC28* (AF 0.533) and a pathogenic missense mutation in p.N626I *SF3B1* (AF 0.111) that have not been described previously in the literature for UM.

Further studies are necessary to understand the functional significance of mutations in the splicing factor *SF3B1* and *TTC28*, which regulates the progression of mitosis and cytokinesis.

Microsatellite Analysis of Irradiated and Non-Irradiated Uveal Melanoma; Assessing Metastasis-Free Survival and Genotype Success.

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Radiotherapy (RXT) is a highly effective modality of treatment in the management of uveal melanoma (UM). Patients undergoing RXT are often offered diagnostic/prognostic biopsies enabling histological and genetic analysis of the tumours. Microsatellite analysis (MSA) of UM is commonplace for low DNA yielding biopsy samples. 284 UM cases genotyped by MSA including the largest cohort to date of UM samples obtained before or after RXT were examined. The findings were correlated with genetic, histopathological,

clinical data and metastasis-free survival.

DNA was extracted and genotyped using MSA (4 microsatellite loci on 3p and 4 microsatellite loci on 3q). Samples were categorised as being either: monosomy 3 (M3), disomy 3 (D3), partial loss, allelic imbalance (AI) or unclassifiable. Analyses were carried out using SPSS Statistics v.24.

78 UM were M3, 174 D3, 8 had loss of 3q, 17 AI and 8 were unclassifiable. Both M3 (Log rank; p=<0.001) and loss of 3q (Log rank; p=<0.001) were significantly associated with a reduced metastasis-free survival. AI were most similar to patients with D3 in terms of metastasis-free survival (Log rank; p=0.480). 240/284 (85%) were biopsies; 128 were taken before RXT and 112 after, with no significant difference in success rate of chr. 3 classification (Fisher's exact p=0.710). Survival for patients who had samples taken either before or after administration of RXT and was similar between both groups (Log rank; p=0.413).

MSA accurately determines chr. 3 status in small UM biopsy samples with low DNA concentrations and reliably establishes chr. 3 status in post RXT samples. Partial loss of chr. 3 is an indicator of poor prognosis, and allelic imbalances are similar to D3 in terms of patient survival. There was no increased incidence of metastasis in patients for whom biopsy was undertaken pre-RXT.

BET proteins inhibition to optimize BRAFi/MEKi therapy for mutant BRAF melanoma

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Mutant BRAF melanoma patients respond rapidly to BRAF and MEK inhibitors (BRAFi/MEKi), but only a minority will achieve a complete response. One mechanism that contributes to the heterogeneous response to BRAFi/MEKi is the adaptive upregulation of several receptor tyrosine kinases (RTKs). While co-treatment with individual RTK inhibitors enhance BRAFi/MEKi targeting effects, therapeutic strategies that broadly target multiple RTKs may achieve more durable effects. Recent studies have shown that bromodomain and extra-terminal family proteins inhibitors (BETi) decrease tumor growth and survival that is associated with RTK downregulation; however, the use of first generation of BETi has been limited by toxicity. In TCGA dataset, we identified that high expression of BRD2 and BRD4 is associated with poor overall survival in Stage III melanoma, and BRD4 is positively correlated with the expression of the RTK ErbB3 in mutant BRAF melanoma. We hypothesized that BETi will improve the efficacy of BRAFi/MEKi, in part, by blocking RTK upregulation, and an intermittent treatment will improve toxicity effects. Hence, we tested the effects of the novel BETi, PLX51107, to block RTK upregulation and improve the efficacy of BRAFi/MEKi. Here, we showed that the BETi by JQ1 and PLX51107 or BRD2/4 knockdown reduced BRAFi/MEKi-associated upregulation of RTKs expression, PDGFR-b and ErbB3. BETi also inhibited paracrine activation of ErbB3 induced by cancer associated fibroblasts (CAFs)-secreted ligand, NRG1. Enhanced/prolonged effects on tumor growth inhibition were observed in vitro, and in human skin reconstructs, driving a durable complete response in mice treated with that intermittent schedule of the triple combination of BRAFi/MEKi/BETi, following cessation of treatment. Thus, our data suggest the intermittent inhibition of BET proteins will improve the efficacy of BRAFi/MEKi in mutant BRAF melanoma.

Comparative efficacy and safety of nivolumab (NIVO) versus other treatments for resected melanoma in adults: A systematic literature review (SLR) and network meta-analysis (NMA)

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The study objective was to evaluate the efficacy and safety of NIVO versus ipilimumab (IPI), pembrolizumab (PEM), combination dabrafenib and trametinib (DAB+TRAM), and interferons (IFNs) in the adjuvant treatment of non-metastatic melanoma in adults by means of an NMA.
The SLR identified 25 RCTs; 19 were included in the analyses after the feasibility assessment. Recurrence-free survival (RFS) was reported in all studies and reflected the primary efficacy outcome of interest. Grade 3/4 adverse events and treatment discontinuations were also analyzed. All analyses were synthesized by means of Bayesian NMAs. Analyses of RFS were conducted assuming both constant and time-varying hazard ratios (HRs).
Results assuming a constant HR suggest NIVO was associated with a statistically significant reduction in risk of recurrence when compared with all other treatments except PEM (HR 0.88, 95% credible interval [CrI] 0.62, 1.24) and DAB+TRAM (HR 1.07, 95%CrI 0.77, 1.48). Results from the time-varying analyses were similar for all comparisons except NIVO versus either DAB+TRAM or PEM. When compared with DAB+TRAM, NIVO was associated with a reduction in risk of recurrence over time; HRs were 5.25 (95%CrI 2.82, 10.46) at 3 months and 0.26 (95%CrI 0.12, 0.56) at 30 months. When compared with PEM, NIVO was associated with a statistically significant reduction in risk of recurrence at one month (HR 0.31, 95%CrI 0.10, 0.93) and was comparable beyond one month. NIVO had a favorable safety profile compared to all active treatments. Overall, NIVO provides an important adjuvant treatment

option for melanoma with a promising risk-benefit profile. The availability of longer term survival data may provide more certainty in a comparison of NIVO and DAB+TRAM.

Mitfa[low] zebrafish melanomas reveal transcriptional signature subdivision and lineage-independent subpopulations *in vivo*

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The MITF-low transcriptional category categorized by TCGA is represented across all genomic subtypes of human cutaneous melanoma, but little is known about the significance of this transcriptional classification. Here we use a conditional *mitfa* allele to show that a Mitfa[low] mutation in zebrafish co-operates with a p53 mutation to promote melanoma independently of *RAS*, *BRAF* or *NF1* mutations. Mitfa[low]p53 melanomas express immune and canonical Wnt gene signatures. An additional *BRAF*[V600E] mutation accelerates the onset of melanoma initiation, and leads to two melanoma subtypes characterized by either a novel neuronal and cilia transcriptional program or a neural crest-Mitf lineage transcriptional program. Complete inhibition of Mitfa activity leads to rapid tumor regression in all models, but once Mitfa activity is restored melanomas recur at the same site as the original tumor. This suggests that a subpopulation of cancer initiating cells remains *in situ* following melanoma regression and is capable of repopulating the tumor. Here, we discover distinct cell subpopulations that persist at the tumor regression site and can be visualized in living fish using fluorescent reporter transgenic lines. We discover a Mitfa[no] melanoma subpopulation that has a distinct transcriptional program compared to the primary melanoma; single cell sequencing suggests that this subpopulation may be present in the tumor prior to melanoma regression. Mitfa[no] cells are enriched for several signaling pathways including ECM and EMT related pathways, suggesting that these cells may contribute to remodeling of the microenvironment and/or melanoma invasion. Our findings suggest that Mitfa[Low] transcriptional activity contributes to melanoma within different genetic contexts, and reveal genetic lineage-independent melanoma cellular heterogeneity *in vivo*.

Investigating the oncogenic properties of RND3 in BRAF[V600E]-driven melanoma

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RND3 is an atypical Rho GTPase that functions in regulating actin cytoskeletal dynamics, and its expression has been shown to be induced by oncogenic RAF signaling. Here, we show that RND3 is expressed in a panel of BRAF[V600E]-driven human and mouse melanoma cell lines and is inhibited by pharmacologic BRAF and/or MEK inhibition. Loss of RND3, via CRISPR-mediated knockout, reduced cell migration and invasion, confirming prior studies. In addition, RND3 knockout cell lines had an approximately two-fold decrease in proliferation, suggesting that this protein may play an important role in melanoma cell cycle. Furthermore, single agent inhibition of BRAF or combined inhibition of BRAF and MEK resulted in enhanced cytotoxicity in RND3 knockout cells compared to wildtype. Thus, elucidating the mechanisms by which this occurs may inform future combination strategies to enhance the efficacy of pathway targeted therapy. In addition, we seek to further elucidate RND3 function in melanoma proliferation, metastasis, and targeted therapy response *in vivo* using syngeneic transplant and genetically engineered mouse models.

Dynamic changes in peripheral T cell populations predict response to PD-1 immunotherapy

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Immunotherapy has revolutionised melanoma treatment, but most patients still die of disease. Blood-based biomarkers to identify check-point inhibitors (CPI) response early during treatment will provide useful and easily accessible guidance for clinical decisions. We analysed peripheral blood mononuclear cells (PBMCs) and plasma cell-free DNA (cfDNA) of 26 stage IV melanoma patients before (before the 1st dose, T0) and during (at week 3, W3) therapy with 1st line CPI (anti-PD1 or anti-PD1 plus anti-CTLA-4 drugs) to study circulating T lymphocyte subsets and T cell receptor beta (TCRβ) repertoire. Responses were determined at 12 weeks (W12) as per standard practice.
In PBMCs, we used high dimensional flow cytometry to quantify 20 T cell subpopulations and analysed if their changes over time were prognostic. We found that a CD8+ T cell subpopulation (Teff) expanded at W3 in patients who then responded to therapy (N=13), but not in patients who progressed (N=13) (P<0.001). In a subgroup of patients we analysed the pattern of T cell turnover, which is also indicative of T cell clearance. Assessed indirectly by means of cfDNA TCRβ sequences, we found that T cell turnover correlated with Teff expansion (P=0.013).

Finally, we studied the evolution of TCRβ repertoire in PBMCs after one dose of CPI, comparing T0 and W3. We developed an algorithm (linear discriminant analysis) based on the changes that occurred in TCRβ repertoire during the first 3 weeks of treatment, and segregated the patients who then achieved disease control from those who progressed at W12 (P=0.007). The algorithm was externally validated (accuracy 85%) using publically available sequencing data.

In conclusion, we propose promising blood-derived biomarkers to identify, at 3 weeks on treatment, patients who will respond to immunotherapy. These biomarkers are candidates for clinical development to assist clinical decisions.

Importance of *SPRED1* in melanoma

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Neurofibromin and SPRED1 are both important negative regulators of the RAS-MAPK signaling pathway. SPRED1 recruits neurofibromin to the plasma membrane to downregulate activated RAS. The *NF1* gene is a known key player in the development of cutaneous melanoma (CM); however, the role of *SPRED1* in CM remains to be clarified. We will test the hypothesis that *SPRED1* is a key tumor suppressor gene in a subset of human CM. According to The Cancer Genome Atlas, a *SPRED1* mutation is found in 5% of CM and patients with high *NF1* expressing melanomas have a better overall survival than patients with low *NF1* expressing melanomas. The same holds true for *SPRED1*. Moreover, preliminary data show that a conventional *Spred1* knockout results in a strong hyperpigmentation phenotype in mice, similar to conditional *Nf1* knockout mice. At the moment CMs are genomically classified into 4 major groups: *BRAF* subtype, *RAS* subtype, *NF1* subtype and triple wild-type subtype. We hypothesize that *SPRED1* mutated melanomas, which are currently classified in the triple wild-type group, phenocopy and belong to the same functional group as *NF1* mutated melanomas. Mutation analysis was performed on a large cohort of frozen human melanoma samples (n=109) with the HaloPlex Noonan Spectrum Syndrome kit, which screens 13 genes of the RAS-MAPK pathway including *SPRED1* and *NF1*. The presence of copy number variations in the *NF1* or *SPRED1* gene was examined using multiplex ligation-dependent probe amplification. The genetic profiles of these samples were compiled. Based on these results we will investigate whether *SPRED1* inactivated CMs present clinically different than *NF1* inactivated CMs. In addition, melanoma mouse models are being generated to genetically ablate *SPRED1* during melanomagenesis and to study the consequences of this inactivation. With this approach, we will further elucidate the role of *SPRED1* in CM and investigate whether this role is equivalent to the role of *NF1*.

RPS3 fucosylation in melanoma

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Fucosylation is reduced in progressive melanoma and increasing fucosylation slows tumor growth and decreases metastatic burden. Despite these observations, the proteins or pathways that are influenced by fucosylation and therefore contribute to tumor growth and metastasis are unknown. The goal of this work is to identify fucosylated proteins in melanoma cells and to characterize the role their fucosylation plays in melanoma growth, progression and response to therapy.

We identified fucosylated proteins in melanoma cells through two methods. First, a screen for all fucosylated proteins was performed using click-chemistry isolation of proteins labeled with modified fucose followed by LC-MS/MS identification. A second screen for fucosylated proteins was performed by lectin pull-down followed by LC-MS/MS identification.

We identified over 200 unique proteins as being fucosylated in melanoma cells. A subset was selected for validation and further exploration. Ribosomal protein S3 (RPS3) was found to be fucosylated in multiple cell types. RPS3 protein is elevated in malignant cells and RPS3 expression confers behavior associated with metastatic disease. RPS3 is localized to both the cytoplasm and nucleus in melanoma cells; however, fucosylated RPS3 is localized almost entirely to the cytoplasmic fraction where it associates with proteins involved in RNA biology. Fucosylated RPS3 binds to nearly 4000 RNAs from several sub-groups of RNA species, including both coding and non-coding transcripts. Treatment with front-line therapeutic agents (*e.g.* Vemurafenib/Trametinib) leads to alterations in RPS3 fucosylation that coincide with changes in its interaction with RNA and RNA-binding proteins. These findings suggest that RPS3 and the post-translational fucosylation of RPS3 may contribute to melanoma progression and have a role in the cellular response to therapeutic stress through a novel mechanism involving RNA splicing, trafficking, and stability.

The landscape of driver mutations in cutaneous melanoma

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Past sequencing studies revealed cutaneous melanoma has one of the highest mutation burdens, which has impeded the discovery of driver events in this cancer. Published power calculations reported that thousands of exomes are needed for analysis to identify genes

possessing driver mutations in >2% of melanoma cases. The TCGA reported 13 high-confidence significantly mutated genes (SMGs) from an analysis of 318 exomes, and proposed cutaneous melanoma could be categorized into 4 genetic subtypes: *BRAF*, *RAS*, *NF1* and *Triple Wild-Type* (*TWT*). To better define the landscape of driver mutations we performed the largest mutation significance study of melanoma, analyzing over 1,000 exomes from five separate studies. Our analysis revealed new infrequently mutated tumor suppressors, frequently mutated epigenetic regulators not previously reported to be significantly mutated, and a X-linked SMG exclusive to male patients. To probe relationships between SMGs and transcriptional subgroups, we applied non-negative matrix factorization to the melanoma TCGA RNA-Seq dataset. TCGA previously identified three mRNA subgroups in 333 melanomas defined by immune, keratin and low MITF expression. Performing clustering analysis of RNA-Seq data on 469 cases, we report two additional intrinsic melanoma subgroups in the TCGA dataset characterized by oxidative phosphorylation and SMARC2 target-gene expression signatures. Notably, a subset of newly identified SMGs was upregulated in the oxidative phosphorylation subgroup. To functionally validate candidate driver mutations, we established mouse models of the four genomic subtypes (*BRAF*, *NRAS*, *NF1*, *TWT*) that utilize Cre-Lox technology and express Cas9 in melanocytes, enabling *in vivo* genome editing to more rapidly generate pre-clinical models of melanoma. In summary, we present the largest mutation significance analysis in melanoma to clarify the landscape of driver events in this disease.

A Randomized, Open-Label, Phase 2, Open-Platform Study Evaluating the Efficacy and Safety of Novel Spartalizumab (PDR001) Combinations in Previously Treated Unresectable or Metastatic Melanoma (PLATforM)

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Significant advancements, including development of immune checkpoint inhibitors and targeted therapies, have transformed outcomes in patients (pts) with unresectable or metastatic melanoma. However, pts who do not respond or who progress while receiving these regimens have limited options. Spartalizumab is a high-affinity, humanized monoclonal antibody blocking the programmed cell death-1 (PD-1) receptor. This randomized, open-label, 2-part, multicenter, open-platform, phase 2 study (NCT03484923) will evaluate safety and efficacy of spartalizumab combination treatment in pts with unresectable or metastatic melanoma progressing after prior anti–PD-1/L1 therapy and, if the tumor harbors a *BRAF* V600 mutation, a BRAF inhibitor. The primary endpoint is objective response rate in each arm per RECIST v1.1; secondary endpoints include duration of response and assessment of paired tumor biopsies for biomarkers of antitumor T-cell activity. The first "selection" part will begin with 3 combination arms: (1) spartalizumab + LAG525 (LAG-3 antibody), (2) spartalizumab + capmatinib (c-MET inhibitor), and (3) spartalizumab + canakinumab (IL-1β antagonist). An adaptive design will allow dropping arms for futility, adding new arms, and selecting ≥ 1 arm for expansion. Bayesian methodology will be used with specific probability criteria for futility and efficacy assessments at each interim analysis. Pts (≈ 60-85) will be stratified by baseline lactate dehydrogenase and randomized equally to all open arms during the selection part. In the second "expansion" part, efficacy and safety of treatment combination(s) selected during part 1 will be further investigated. Sample size for part 2 will be adaptive and based on predictive power calculations considering the results from part 1.

Paradoxical role for wild type p53 in driving therapy resistance in melanoma

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Metastasis is the major cause of human cancer deaths. In many types of cancer, pathways that promote metastasis have been linked with therapy resistance. In melanoma, the non-canonical Wnt signaling pathway, which drives an invasive phenotype, promotes

resistance to targeted therapy via Wnt5A. Our previous data suggest that Wnt5A promotes a senescent-like phenotype, which has many of the hallmarks of senescence, but the cells remain highly invasive and retain the ability to form colonies. In the current study, we show that Wnt5A drives a slow-cycling phenotype via wild type p53. Knocking down Wnt5A or p53 decreased the percentage of slow-cycling cells and the addition of rWnt5A to the p53 knockdown cells was unable to rescue the slow-cycling phenotype. When we isolated these slow-cycling cells, we found that they have an increase in Wnt5A and p53 expression. p53 is responsible for cellular response to multiple types of stress, including oxidative stress, which we have previously shown to be increased in an aging microenvironment. Here, we found that tumors grown in aged mice have an increase in Wnt5A and p53 expression, which correlates with an increase in a slow-cycling phenotype in vivo. Analysis of patient tumors revealed that older patients between 45-69 years have significantly higher probability to have more than 3% of their tumor positive for p53 compared to younger patients (<45 years). We then asked if inhibiting p53 could promote sensitivity to BRAF/MEKi therapy. We found that treating mice with a single dose of a p53 inhibitor promoted sensitivity to BRAF/MEKi therapy. Analysis of PDX tumors, revealed an increase in p53 expression following BRAF/MEKi therapy. These data suggest that these slow-cycling cells driven by Wnt5A and p53, which are enriched following multiple types of stress including targeted therapy and aging, drive therapy resistance in melanoma.

Time on Treatment with Pembrolizumab in 665 Advanced Melanoma Patients in a Real World Setting

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Immune checkpoint inhibition using antibodies against Programmed-Death-1 receptor functioning has created a whole new class of successful cancer therapies. Advanced melanoma is among the pivotal tumors demonstrating high rate of long-term survivors in a previously hard-to-treat condition. We evaluated real world time on treatment (rwToT) in a large cohort of pembrolizumab treated patients in Germany.
Patients initiating pembrolizumab from Aug 2015 to Dec 2017 for unresectable stage III or stage IV melanoma were examined from the German national skin cancer registry (ADOReg). rwToT was calculated as the interval between first and last dose of pembrolizumab. Kaplan-Meier estimates for median rwToT and 1-year on-treatment rate were generated.
In total we evaluated 665 pembrolizumab patients with non-resectable stage III (n=65) or stage IV (n=600) melanoma. Of these, 387 were treatment-naive and 278 were pretreated with chemotherapy, BRAF inhibitor, or CTLA-4 blockade. In addition to cutaneous melanoma (n= 539), the study population consisted of ocular (n=30), mucosal (n=16), and unknown (n=80) origin of melanoma. In first-line, the median rwToT was markedly longer than in second-line (209 days vs. 148 days). However, in third-line or higher the median rwToT was 190 days. This was also reflected by 1-year on-treatment rates of 34.4%, 28.9%, and 30.1% in first-, second-, and third-line plus, respectively. Median rwToT was longest for unknown primary (228 days) and cutaneous melanoma (203 days), but markedly shorter for ocular (84 days) and mucosal (106 days) melanoma. In contrast, the presence vs. absence of brain metastasis did not affect median rwToT (189 vs. 196 days).
In conclusion, rwToT with pembrolizumab was reasonably long, even in patients receiving the treatment in third or higher line. The presence of brain metastasis did not result in shorter rwToT.

Members of the PI3K/AKT/mTOR pathway are differentially methylated in melanoma intra- and extracerebral metastases

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Around 75% of stage IV melanoma patients develop brain metastases during the course of their disease. Although targeted and immunotherapies achieve similar initial response rates in intra- and extracerebral metastases, the majority of patients eventually progress and die from new brain metastases, indicating brain-specific resistance mechanisms. Uncovering the mechanisms responsible for the different behavior of the intra- and extracerebral metastases will thus greatly aid in the search for more brain-specific therapeutics. Initial studies could not detect any significant hotspot mutations or copy number variations distinguishing intra- from extracerebral metastases, but observed epigenetic reprogramming of melanoma brain metastases facilitated by the brain microenvironment. We thus wanted to analyze the methylation and messenger RNA expression differences of intra- and extracerebral metastases and performed a methylation array and RNA-Seq on FFPE material of 19 matched pairs. Using integrative bioinformatics approaches, we identified 34 candidates that were differently methylated in the intra- and extracerebral metastases and had a corresponding RNA expression level response. Additionally, over 100 candidates from the RNA-Seq data were de-regulated in the intra- compared to extracerebral metastases. Many of the candidates belong to signaling pathways shown to be important for survival and metastasis formation of different cancers, such as cell cycle, metabolic pathways, TGF-beta, MAPK, PI3K/AKT and mTOR signaling pathways. Notably, recent studies by others and our lab showed a hyperactivation of the PI3K/AKT/mTOR pathway in intra- compared to extracerebral metastases. We will focus on validating the candidate genes from this pathway by immunohistochemistry and functional assays to elucidate their role in the resistance of brain metastases to therapy.

Retroviral Reactivation as a Therapeutic Target in Melanoma
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Despite huge advances in current therapies, the prognosis for patients with late stage malignant melanoma is poor, with a five-year survival rate of 5-19%. Preclinical studies have demonstrated synergy when combining MAPK-targeting with immune based therapies. However, it is not clear how this will translate into the clinical setting. Our studies aim to elucidate the mechanisms through which MAPK inhibition might be able to further sensitise melanoma to treatment with immunotherapies. Preliminary results show activation of a type I Interferon response in melanoma cells after short-term treatment with a MEK inhibitor. This induction correlated with increased expression of the RNA and DNA sensors involved in an antiviral response and a subsequent increase in *IFNß* expression. In searching for factors, which may underlie this anti-viral response, we found increased expression of human endogenous retrovirus (HERV) RNA in melanoma cells after short-term treatment with both BRAF & MEK inhibitors. This induction of HERV expression was linked to the melanoma transcriptional master-regulator MITF, which itself is a target of the MAPK-pathway. Because activation of a type I interferon response has been linked to an improved anti-PDL1 response, targeting MAPK/MITF driven HERV expression could therefore contribute to sensitization of melanoma to immunotherapy.

Outcome of Chinese Melanoma after Lymph Node Dissection: A Single Center Experience
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Backgrounds:
MSLT-1 trial demonstrated an outcome benefit in disease-free survival (DFS) but not in overall survival (OS) for clinical stage I/II cutaneous melanoma underwent sentinel node biopsy (SNB) compared with observations. However, MSLT-2 trial showed no advantage in either DFS or OS to do further complete lymph node dissection（CLND）. This study aims to explore the impact of CLND on Chinese melanoma.

Methods:
We retrospectively collected melanoma patients underwent CLND and pathologically proved to have lymph node metastasis from Melanoma Database Pool in Fudan University Shanghai Cancer Center. All treatment protocols were supervised by the Ethics Committee Board, and relative consent forms were obtained individually. Comparison of survival outcomes were computed by Kaplan–Meier method.

Results:
Total 459 patients were recruited in our study. 111 patients received CLND after SNB, while 348 patients received a therapeutic CLND (TLND) until clinical metastasis observed. Mean Breslow thickness in two cohort was 4.09mm and 4.77mm respectively (P=0.588). The TLND group had more mucosa cases (P<0.001) and higher ulceration rate (87.6% vs 75.6%, P=0.044). Meanwhile in this group, patients apparently had more involved nodes after dissection, compared with those with micro-metastasis tested in SNB(mean no. of positive LN 2.94 vs 0.45, P<0.001). With a median follow-up of 29 months, immediate CLND after a positive SN only brought benefit in PFS (33 months vs, 20 months, P=0.018), but not in OS (72 months vs 74 months, P=0.437), compared to delayed TLND.

Conclusion:
Results of our study seems to be aligned with MSLT-2 study. Even with a more severe tumor burden in Chinese melanoma population, CLND might not improve overall outcomes patients with positive SN, which implies that lymphatic and hematogenous metastasis can occur simultaneously in thick cutaneous melanoma and mucosa melanoma.

Bi-allelic loss of *CDKN2A* initiates melanoma invasion via BRN2 activation
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CDKN2A acts as a critical tumor suppressor in melanoma, as evidenced by frequent loss of function mutations and deletion. Loss of *CDKN2A* is believed to permit escape from senescent pre-neoplastic cell populations through relieve of a cell cycle block mediated by its two gene products. We performed a comprehensive analysis of *CDKN2A* gene status, mRNA and protein expression levels of p16^{INK4A} and p14^{ARF} in a cohort of melanomas and their adjacent pre-neoplastic lesions and observed that bi-allelic *CDKN2A* loss coincides with the progression stage when primary melanomas become invasive. In melanoma lines, p16^{INK4A}, one of the protein products of the *CDKN2A* locus, is a potent barrier to metastasis, independent of its known role inhibiting cell proliferation. We genetically engineered primary human melanocytes to harbor *CDKN2A* deletions and/or *BRAF^{V600E}* mutation at their endogenous BRAF locus. Using this physiologic model for the early phases of neoplastic transformation, we found no evidence for BRAF-induced senescence, rather observing that p16^{INK4A} loss activates a master regulator of melanoma invasion, BRN2, through Rb-E2F1 axis. These results demonstrate that one of the most frequently altered genes across human cancers, *CDKN2A*, has an unexpected novel role in inhibiting cellular invasion through lineage specific transcription factors and acts as an essential gatekeeper of early metastatic dissemination.