

TITLE: Functional Characterization of Novel Melanoma Oncogenes and Tumor Suppressors Utilizing a CRISPR/Cas9 Mouse Model

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ABSTRACT BODY:

Body: Genetically Engineered Mouse (GEM) models have been indispensable tools to validate the function of melanoma-associated genes. Mouse models served as important preclinical tools in the development of anti-CTLA-4 and anti-PD-1 immune checkpoint inhibitors that have produced dramatic changes in melanoma patient outcome. However, limitations associated with developing GEMs are the cost and time required to produce these models. In addition, next generation sequencing studies have identified many new tumor suppressors and oncogenes in melanoma making the systematic creation of GEMs targeting these genes using traditional approaches a challenging endeavor. With the advances in genome editing technology, we now have the ability to more rapidly modify the DNA of any organism. Herein, we present our preliminary in vivo and ex vivo studies utilizing CRISPR/Cas9 technology to generate pre-clinical mouse models of melanoma.

TITLE: Prognostic and predictive value of AJCC-8 staging in the phase 3 EORTC 1325/KEYNOTE-054 trial of pembrolizumab vs placebo in resected high-risk stage III melanoma

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ABSTRACT BODY:

Body: The AJCC-8 classification of melanoma was implemented in January 2018. It was based on data gathered when checkpoint inhibitors were not used as adjuvant therapy in stage III melanoma. The EORTC 1325/KEYNOTE-054 double-blind phase 3 trial evaluated pembrolizumab vs placebo in AJCC-7 stage IIIA (excluding lymph node metastasis ≤ 1 mm), IIIB or IIIC (without in-transit metastasis) patients (pts) after complete resection of involved lymph node(s). Patients (n=1019) were randomized 1:1 to pembrolizumab 200 mg or placebo every 3 weeks (total of 18 doses, ~1 year). At 1.25 yr median follow-up, pembrolizumab prolonged relapse-free survival (RFS) in the total population (1-year RFS rate: 75.4% vs 61.0%; HR 0.57, 98.4% CI 0.43-0.74; $P < 0.0001$), and the PD-L1-positive subgroup, and consistently in the AJCC-7 subgroups (Eggermont et al, NEJM, 2018). Prognostic and predictive values of AJCC-8 for RFS were evaluated in this trial. Patient distribution according to the AJCC-8 stage III subgroups was 8% (IIIA), 34.7% (IIIB), 49.7% (IIIC), 3.7% (IIID), and 3.8% (unknown). AJCC-8 classification was strongly associated with RFS (HRs for stage IIIB, IIIC, and IIID vs IIIA were 4.0, 5.6, and 12.1, respectively) but showed no predictive importance for the treatment comparison regarding RFS (test for interaction: $P = 0.68$). The 1-year RFS rate for pembrolizumab vs placebo and the HRs (99% CI) within each AJCC-8 subgroup were: stage IIIA [92.7% vs 92.5%; 0.76 (0.11-5.43)], IIIB [79.0% vs 65.5%; 0.59 (0.35-0.99)], IIIC [73.6% vs 53.9%; 0.48 (0.33-0.70)], IIID [50.0% vs 33.3%; 0.69 (0.24-2.00)]. In this re-analysis, AJCC-8 had a strong prognostic importance for RFS, but no predictive importance: the RFS benefit of pembrolizumab was observed across AJCC-8 subgroups in resected high-risk stage III melanoma patients.

TITLE: Evaluating centrosome-clustering in Uveal Melanoma

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ABSTRACT BODY:

Body:

Background:

50% of all Uveal Melanoma (UM) patients develop metastatic disease, typically to the liver, and the prognosis is poor. Thus, there is a pressing need to develop new strategies to cure metastatic UM.

Treatment of primary UM is successful in most cases, and involves surgery and/or radiation. The latter includes Proton-beam therapy (PBT), yet not all tumours respond. Therefore, there is also a need to find methods to target radiation-resistant UM.

Centrosomes are the dominant microtubule-organising centre in animal cells. At mitosis, 2 centrosomes are present, which anchor the bipolar mitotic spindle. However, centrosome amplification (CA) is common in some cancers. Cells cope with CA by clustering centrosomes into two functional groups, allowing cell division. Certain proteins, notably KIFC1, are required for clustering and, thus, present cancer-specific therapeutic targets.

Of additional interest, CA can be induced by gamma-irradiation. However, whether PBT also causes CA is unknown.

Aims:

We initially observed multipolar mitoses, an indicator of CA, in sections of proton-beam treated UM. This led us to ask two questions:

1. Do UM cells display CA and if so does this sensitize them to KIFC1 inhibition?
2. Does PBT cause CA?

Results:

1. Examination of a cell panel revealed that centrosome amplification is prevalent in UM, particularly in cells derived from metastases. Centrosomes are clustered effectively, suggesting cells are reliant on this process for survival. In agreement with this, metastatic UM cells were equally as sensitive to KIFC1 inhibition as BT549, a well-established breast model of CA.

2. 72hr post-irradiation with high energy/low LET protons (58 MeV), cells displayed a dose-dependent increase in CA. Our data show that CA occurs due to cytokinesis failure caused by DNA damage following irradiation.

Conclusions:

These observations open new potential avenues of therapeutic intervention in UM.

TITLE: Combined inhibition of AXL and the DNA damage response as a therapeutic strategy in melanoma

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ABSTRACT BODY:

Body: The receptor tyrosine kinase AXL is found upregulated in melanomas and correlates with an aggressive cancer phenotype, inducing cell proliferation, epithelial-to-mesenchymal transition and immune suppression. In addition, AXL has been found to promote chemotherapy resistance and inhibition of AXL reduces expression of proteins involved with the DNA damage response. In light of this, we hypothesized that targeting AXL in combination with DNA damage response proteins would be beneficial in melanomas. Thus, we aimed to combine inhibition of AXL in combination with inhibition of the serine/threonine-specific kinases Chk1/2, which are activated in response to DNA damage and coordinates the DNA damage response.

Our data demonstrates that inhibiting AXL (BGB324) and Chk1/2 (AZD7762) in combination synergistically reduced cell proliferation and migration in melanoma cell lines. An increased effect of combined treatment was also observed in an ex-vivo 3D "organoid" assay on disaggregated melanoma lymph node metastases harvested directly from the patients, as well as in animal model. The combination showed deregulation of the cell cycle and increased apoptosis compared to monotreatments. Both BGB324 and AZD7762 monotreatments showed increased DNA damage through inhibitory phosphorylation of mitotic inducer protein Cdc25C. However, cell cycle regulation proteins, such as Chk1, Chk2 and Cdc25C were downregulated in the combined treatment. As treatment with a Chk1 specific inhibitor (LY2603618) did not demonstrate reduced proliferation, either alone, or in combination with AXL inhibition, the observed effects of AXL and Chk1/2 inhibition is most likely mediated through Chk2.

Together, our results demonstrate that combined targeting of AXL and the DNA damage response pathway may be a novel treatment strategy in melanoma.

TITLE: Genomic events targeting the antigen presentation genes in melanoma

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ABSTRACT BODY:

Body: The immune system can recognise tumours via the display of tumour-specific fragments by MHC-class I or –class II molecules. Tumours can evade immune recognition by alteration of genes or chromosomal regions encoding the MHC molecules and the antigen processing machinery to alter antigen display. While antibodies can detect alteration in overall expression on cell surfaces, few can specifically detect individual HLA subtypes.

We employed an integrative approach of several molecular techniques, including, SNP array, targeted HLA sequencing, whole exome/genome sequencing, methylation array and RNAseq, to assess aberration of the HLA region in melanoma. In 199 melanoma cell lines and the SKCM cohort from TCGA, we have shown one third of samples had no HLA aberration and one third had a gain of 6p (and a reciprocal loss of 6q). The call of loss of heterozygosity (LOH) using SNP array was confounded by germline homozygosity in the HLA allele, indicative of strong haplotype linkage disequilibrium across the genes. Compound alterations were observed including LOH of one allele and nonsense mutation in the second. LOH-independent hemizygous methylation of the promoter of HLA-A and –B was associated with decreased RNA expression. Finally, deep deletion in the MHC-class I support molecule β_2M , as well as compound LOH and nonsense mutation in β_2M and CIITA revealed another mechanism by which HLA antigen display is targeted in melanoma.

Our results indicate that careful integration of molecular data should be undertaken to reveal the spectrum of possible aberrations impacting a specific HLA-allele, or entire HLA-gene in melanoma. Given the recent resurgence of interest in personalised immunotherapy, we conclude that it is vital to fully assess the molecular features of the HLA genes in the tumour, before undertaking an individualised therapeutic approach.

TITLE: SWATH mass spectrometry reveals the diverse proteomic landscape of mucosal melanoma

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ABSTRACT BODY:

Body: Mucosal melanoma is usually considered the most aggressive melanoma subtype, accounting for 22.6% of melanomas in Asians. In this study, we aim to obtain the comprehensive proteomic view of this subtype of melanoma arising from different primary anatomic sites. 39 fresh-frozen tumors arising from female genital (13 cases), nasal (13 cases) and oral (13 cases) mucosa were performed the proteomic analysis through data-independent acquisition (DIA) mass spectrometry. We identified hundreds of proteins were significantly up or down-regulated among the three cohorts. Differentially expressed proteins were enriched in pathways associated with cellular process, single-organism process and biological regulation, etc. More interestingly, there is an expression difference of CDK4 among the three cohorts, a gradual increase trend in the genital, nasal and oral melanoma cohort. This result was validated in an independent extension cohort via IHC analysis. Then, we analyzed the sensitivity of patient-derived xenograft (PDX) models to CDK4/6 inhibitors and identified the CDK4/6 inhibitor abrogated tumor growth in the nasal and oral melanoma models with CDK4 overexpression. Thus, our study reveals the distinct proteomics and potentially different therapeutic targets of mucosal melanoma arising from different primary anatomic sites and sheds lights to the further personalized medicine for this subtype of melanoma patients.

TITLE: Pre-operative ctDNA predicts poorer survival in high risk AJCC stage III melanoma patients.

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ABSTRACT BODY:

Body: Introduction

The outcomes of patients with stage III melanoma who undergo complete surgical resection can be highly variable, and estimation of individual risk of disease relapse and mortality remains imprecise. With recent demonstration of effective adjuvant targeted and immune checkpoint inhibitor therapy, more precise stratification of patients for costly and potentially toxic adjuvant therapy is needed. We report the utility of pre-operative circulating tumour DNA (ctDNA) in patients with high risk stage III melanoma.

Patients and methods

ctDNA was analysed in blood specimens that were collected pre-operatively from 246 patients with stage III melanoma undergoing complete lymph node (LN) dissection. Cox regression analyses were used to evaluate the prognostic significance of ctDNA for distant metastases free survival and melanoma specific survival.

Results

The detection of ctDNA in the discovery and validation cohort was 34% and 33% respectively, and is associated with larger melanoma deposit, higher number of melanoma involved LNs, more advanced stage and high lactose dehydrogenase (LDH) levels. Detectable ctDNA was significantly associated with worse melanoma specific survival (MSS) in the discovery (hazard ratio (HR) 2.11 p = 0.002) and validation cohort (HR 2.36, p = 0.033) and remained significant in a multivariate analysis (HR 2.14, p = 0.036). ctDNA further substratified patients with AJCC stage III substage, with increasing significance observed in more advanced stage melanoma; HR for ctDNA detectable versus undetectable in stage IIIC/D melanoma 3.1 (95% CI 1.56 – 5.99, p = 0.0002).

Conclusion

Pre-operative ctDNA can predict melanoma specific survival in high risk stage III melanoma patients undergoing complete LN dissection, independent of stage III subclass. This biomarker may have an important role in prognosis and stratifying patients for adjuvant treatment.

TITLE:

Long-term follow-up of standard-dose pembrolizumab (pembro) plus reduced-dose ipilimumab (ipi) in 153 patients (pts) with advanced melanoma (MEL): KEYNOTE-029 1B

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ABSTRACT BODY:

Body: The currently approved regimen of reduced-dose anti-PD-1 plus full-dose anti-CTLA-4 therapy has significant efficacy but substantial toxicity in MEL. Cohort B of the phase 1b KEYNOTE-029 study (NCT0289685) assessed the safety and antitumor activity of standard-dose pembro plus reduced-dose ipi in advanced MEL. We present long-term outcomes for this cohort of 153 pts. Eligible pts with advanced MEL, ECOG PS 0-1, and no active CNS metastases received pembro 2 mg/kg (later changed to 200 mg) Q3W + ipi 1 mg/kg Q3W for 4 cycles, then pembro alone for up to 2 y. Endpoints were ORR, PFS, and DOR per RECIST v1.1 by central review, OS, and safety. Median age was 60 y, 66% were male, and 27% had ECOG PS 1, 56% stage M1C disease, 37% BRAF mutation, 25% elevated LDH, and 13% 1-2 prior therapies for advanced MEL. As of 17 Jul 2018, median follow-up was 36.8 mo. 72% of pts received all 4 ipi doses and 31% completed 2 y of pembro; 26% of pts completed both pembro and ipi. ORR was 62% (95% CI 54-70), including 42 (27%) CRs and 53 (35%) PRs. Median DOR was not reached (range 1.6+ to 38.2+ mo); 36-mo DOR rate was 84%. Median PFS and OS were not reached; 36-mo rates were 59% and 73%, respectively. Drug-related AEs (DRAEs) occurred in 96% of pts; 47% were grade 3-4, with no deaths. DRAEs led to discontinuation in 33% of pts: pembro and ipi in 14%, pembro only in 10%, and ipi only in 8%. The most common grade 3-4 DRAEs were lipase increased (18%), autoimmune hepatitis (7%), colitis (5%), and amylase increased (5%). Immune-mediated AEs and infusion reactions occurred in 61% of pts; 26% were grade 3-4. Evaluation of pembro 200 mg Q3W plus alternate ipi dosing strategies is ongoing. Standard-dose pembro plus reduced-dose ipi has robust antitumor activity, favorable long-term PFS, OS, and DOR with manageable safety.

TITLE: Intestinal Microbiome Metabolomics: a Biomarker for Immune Toxicities

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ABSTRACT BODY:

Body: Background: Immune related toxicities (irAEs) from checkpoint inhibitors (ICi) can significantly impact a patient's treatment course, potentially requiring the use of immunosuppressing agents and cause delays in therapy. The composition of the intestinal microbiome, including the virome, contributes to the development of auto-immune disorders. We evaluated pathways known to be involved in the innate immune response such as the RIG-I-like receptors (RLRs), could be predicted to have a larger functional content in patients who develop severe irAEs. This could identify potential biomarkers to identify the risk for irAEs. Methods: We collected stool samples from 29 pts with stage 3-4 melanoma at the University of Colorado prior to and for some after starting ICi therapy (anti-CTLA-4 antibody, anti-PD-1 antibody or the combination). Patients were classified as having a severe toxicity (T) or no severe toxicity (NT); defined as a grade 3-4 toxicity based on CTCAE. We conducted 16s rRNA gene sequencing then used PICRUSt to evaluate metagenome functional content. Results: Non-significant differences in diversity and composition of the gut microbiome were noted in T vs NT, with slightly higher diversity noted amongst T. There was a significant difference in the gene content predictions for the RLRs signaling pathway in the KEGG gene family for T (mean 398.2) vs NT (mean 987.2) $p = 0.005$. Significant differences were also seen in the gene content predictions for the mineral absorption pathway in the KEGG gene family for T (mean 328.3) vs NT (mean 895.8) $p = 0.05$. Conclusions: We observed that the gut microbiome composition may be associated with the potential to develop severe irAEs from immunotherapy. The use of metagenomics may help to identify particular pathways that could predict treatment outcomes. This could have far reaching implications though the results need to be validated in a larger cohort.

TITLE: Abrogation of Autophagy May Improve Antitumor Effects of Arginine Depletion in Melanoma

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ABSTRACT BODY:

Body: Melanoma cells are arginine auxotrophs and often deficient in the arginine synthesis enzyme argininosuccinate synthetase 1 (ASS1). Strategies to target dependence of melanoma on arginine for growth have included the development of compounds to manipulate the function of arginine-degrading enzymes arginine deiminase (ADI) and arginase, with modest antitumor activity reported. The ideal targeting of arginine dependence may be in combination with pathways that promote tumor survival. Tumor cell autophagy plays a role in melanomagenesis in BRAF^{V600E} GEMM melanoma models whose tumors display loss of the autophagy gene atg7. To understand the role of host autophagy, melanoma cell lines were grown in C57BL/6 mice with and without whole body atg7 depletion through an inducible knockout strategy. Tumors from YUMM 1.1 and 1.3, but not YUMM 1.7 or 1.9 demonstrated delayed growth in atg7 deficient mice, indicating that host dependency on autophagy occurs although tumor specific adaptive mechanisms can be present. Tumor cells with delayed growth did not express ASS1 and required arginine supplementation to grow. Serum proteomic analysis identified the arginine degrading enzyme ARG1 in the circulation of atg7 deficient hosts, and in vivo arginine metabolic tracing revealed serum arginine degradation to ornithine. Since ARG1 is expressed predominantly in liver, liver-specific atg7 deletion was pursued. Deletion of atg7 in the liver led to increased circulating ARG1. Reduced circulating arginine and reduced tumor growth were also observed that were partially restored with dietary arginine supplementation. Intact host autophagy thus prevents ARG1 release and arginine degradation, maintaining serum arginine which supports tumor growth. Abrogation of autophagy in the setting of arginine deprivation may be an important strategy to sensitize melanoma to arginine degrading enzymes in development.

TITLE: Multi-platform characterization of cutaneous melanoma from patients treated with immune checkpoint inhibitors

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ABSTRACT BODY:

Body: Therapeutics targeting inhibitory immune checkpoints have revolutionized the treatment of metastatic melanoma. Prior studies have identified tumor mutation burden (TMB), gene expression signatures and tumor aneuploidy as predictors of immunotherapy response, but evidence of associations with mutations in specific genes is still limited. A significant knowledge gap remains regarding which combination of factors best predicts a priori response to immunotherapy. We performed a multi-platform integrative analysis of 38 melanoma samples from patients treated with immune checkpoint inhibitors. This included focused sequencing of >400 cancer-associated genes and DNA copy number profiling using the CANCERPLEX® platform from Kew Group Inc., 7-colour immunofluorescence of immune markers, and analysis of peripheral blood. The focused sequencing panel identified known melanoma driver mutations at frequencies similar to those reported in previously published studies and was used to predict TMB. Lymphocytic infiltration and elevated pre-treatment eosinophil counts were linked to improved treatment response, but we did not observe a significant association with mutated genes or TMB in this cohort of limited size. To increase statistical power, we performed a meta-analysis of publicly available whole-exome sequencing and copy-number data for 315 melanoma patients treated with immune checkpoint inhibitors. Loss of function mutations in two known tumor suppressors, NF1 and ARID2, were globally enriched in immunotherapy responders. High TMB correlated with response in most cohorts, but no association with aneuploidy was found in the subset of patients (n=144) for which absolute copy number data was available. This study adds to a growing body of knowledge to understand the molecular determinants of response to immune checkpoint blockade to further personalize melanoma care.

TITLE: Deconvolution of the innate and adaptive immune infiltrate in highly heterogeneous uveal melanoma

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ABSTRACT BODY:

Body: Uveal melanoma (UM) is the most common primary malignancy of the adult eye, and – after cutaneous melanoma – the second most common subtype of melanoma.

Several prior studies, including a recent TCGA-initiated integrative analysis, identified clinically-relevant UM subtypes. Intriguingly, molecular and cellular heterogeneity largely accounts for the differences observed between tumors.

In a cohort of 63 primary UM samples, DNA/RNA-based tumor profiling was performed and revealed an extensive immune infiltrate in a part of the UM with a poor prognosis. In order to deconvolute this infiltrate, we developed and validated a digital PCR-based T-cell quantification method. Combining the T-cell quantification with cell-type specific transcription patterns, a classically activated immune response in infiltrated UM could be identified. Besides T cells, activated macrophages were shown to be prominently populating the infiltrate. Whereas the T-cell infiltrate itself was not correlated with prognosis, the presence and activation of these macrophages was negatively correlated with metastasis-free survival.

To study the intratumoral adaptive immune repertoire, a selection of primary UM's was analyzed by high-throughput RNA sequencing. Generally, the T-cell receptor (TCR) and immunoglobulin (IG) expression levels correlated with the absolute presence of infiltrating cells. However, large differences in the diversity of the TCR and IG repertoire could be identified between tumors. This suggests that specific immune responses may affect UM progression and that immune therapy may potentially be an option for UM patients.

TITLE: Targeting the CoREST Complex as a Novel Therapeutic Strategy for Melanoma and Overcoming Acquired BRAF Inhibitor Resistance

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ABSTRACT BODY:

Body: The majority of cancers harbor both genetic and epigenetic mutations, and tumor cells routinely use epigenetic processes to ensure escape from chemotherapy and host immune surveillance. Knowledge of this led to the creation of numerous epigenetic agents that are currently being tested for anti-cancer therapy, with several HDAC inhibitors already FDA approved for use in a subset of hematologic malignancies. The major limitation to the widespread use of these epigenetic therapies is the lack of target selectivity and specificity, resulting in a narrow therapeutic window. To combat this, we developed Corin, a small molecule inhibitor of the CoREST chromatin-modifying complex, derived from a class I HDAC inhibitor and an LSD1 inhibitor. Through a screening analysis, we determined that Corin preferentially inhibited growth in human melanoma cells when compared to other cancer cell lines. Cell-based experiments revealed that Corin treatment potently inhibited cell growth across a number of melanoma cell lines and consistently outperformed the anti-proliferative effect of its parent HDAC and LSD1 inhibitors. Furthermore, combination treatment with the parent HDAC and LSD1 inhibitors could not match the anti-proliferative action of Corin. Gene expression analysis revealed that Corin was a more potent inducer of tumor suppressor genes, many of which have been previously observed to be epigenetically silenced in cancer. Corin was also effective in slowing tumor growth in a melanoma mouse xenograft model. Finally, combination treatment with Corin and a BRAF inhibitor restored sensitivity to treatment in BRAF inhibitor-resistant melanoma cells. This dual action inhibitor demonstrates a novel and specific therapeutic approach to targeting epigenetic pathways in human melanoma and may be an important mechanism in overcoming acquired BRAF inhibitor resistance.

TITLE: Cyclooxygenase-2 inhibitors and immune checkpoint blockade therapy synergistically promote tumor control

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ABSTRACT BODY:

Body: Tumors have evolved a plethora of evasive mechanisms to avoid immune recognition and regulation, many of which could constitute immunotherapeutic targets. Upregulation of cyclooxygenase (COX)-2 and downstream production of the inflammatory lipid prostaglandin E₂ (PGE₂) is a common feature of several cancers. We have previously shown that intra-tumoral expression of the COX-2/PGE₂ pathway is associated with a drastic shift in the inflammatory profile at the tumor site, favoring immune evasion and tumor progression. We therefore hypothesized that therapeutically targeting the COX-2/PGE₂ pathway would enhance the efficacy of immune checkpoint blockade against tumors poorly responsive to this treatment modality. Co-administration of systemic anti-PD-1 or anti-PD-1 and anti-CTLA-4 antibody with selective oral COX-2 inhibitors to mice with established tumors resulted in tumor eradication in a large proportion of mice otherwise poorly responsive to either therapy alone. Likewise, anti-PD-1 blockade led to complete responses in mice bearing tumors formed by cancer cells genetically engineered to lack COX-2 expression but not by COX-2-competent control cells. The synergy between immune checkpoint blockade and COX-2 inhibition was conserved across models of melanoma, breast and colorectal cancer suggesting tumor-cell derived PGE₂ is a dominant mechanism of resistance to immune checkpoint blockade. Analysis of the molecular and cellular composition of murine tumours and patient datasets suggest that the COX-2/PGE₂ pathway, in promoting tumour inflammation, limits T-cell dependent tumor control.

In conclusion, our data supports the rationale that COX-2 inhibition could be a means of enhancing the efficacy of immunotherapy in cancer patients.

TITLE: Ultraviolet Radiation Modulates DNA Methylation in Melanocytes

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ABSTRACT BODY:

Body: Epidemiological studies have shown that ultraviolet radiation (UVR) directly causes >80% of melanomas. However, the underlying molecular mechanisms by which UVR promotes melanomagenesis are poorly understood. UVR is incontrovertibly mutagenic; however, the well-studied driver mutations found in a large majority of melanomas (i.e. BRAF^{V600E} and NRAS^{Q61L/R}) do not bear UVR signatures, confounding their melanoma-initiating role. We hypothesized that UVR-induced epigenetic modulations may play an important role in melanomagenesis. Epigenetic control of gene expression through DNA methylation and histone modifications is disrupted during melanomagenesis and dysregulates gene expression of important tumor suppressors and oncogenes; however, it has not been investigated in the context of UVR exposure. Using Digital Restriction Enzyme Analysis of Methylation (DREAM), we demonstrate that UVR modulates DNA methylation in both mouse and human melanocytes in vitro. Multiple UVR exposures enhance this effect. Interestingly, UVR-induced DNA methylation changes significantly overlapped with those found in melanoma, suggesting a potential role in melanomagenesis. UVR-induced epigenetic modulations appear to be functionally relevant to melanomagenesis, as UVR exposure significantly enhanced the tumorigenicity of B16N melanoma cells in vivo. We hypothesize that the initial UVR-induced epigenetic alterations contribute to the susceptibility of a field of melanocytes to melanomagenesis. Our studies provide the first insights into an epigenetic response in melanocytes to UVR exposure, which may have at least a complementary role in UVR-induced melanomagenesis.

TITLE: Involvement of IL-8 Cytokine in the Regulation of Melanoma Cell Growth Based on Curcumin Pre-treatment and IL-8 Treatment of Human Melanoma Cell Models

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ABSTRACT BODY:

Body: Our previous studies showed that progesterone, a female sex hormone inhibited mouse (B16F10) and human (BLM) melanoma cell growth significantly in-vitro. Progesterone action, as shown by Elisarray, was mediated through specific suppression of pro-inflammatory IL-8 cytokine secretion. Further experiment involving direct suppression of IL-8 by curcumin pre-treatment resulted in the decrease of BLM cell growth. In order to check the role of IL-8 in the regulation of melanoma cell growth, experiments were designed 1) to add IL-8 directly to human melanoma cells 2) to carry out dose-response study of curcumin treatment on melanoma cell growth and 3) to rescue cell growth in curcumin pre-treated melanoma cells by adding various concentrations of IL-8. Results indicated that IL-8 (1 ng/ml) stimulated human melanoma (BLM) cell growth to 114% compared to untreated control cell growth at 100%. Curcumin pre-treatment of BLM cells at 10, 25, 50 and 100 μ M showed a dose-dependent decrease of melanoma cell growth. Rescue experiments with IL-8 at 1 ng, 10 ng and 100 ng per ml were carried out after curcumin (10, 25, 50 and 100 μ M) pre-treatment of BLM cells. Addition of IL-8 10 ng/ml to curcumin 25 μ M pre-treated BLM cells at 73% cell growth, rescued BLM cell growth to 86%. All the experiments were repeated with another human melanoma (1205Lu) cell line. Results were very similar to BLM cells, but with a near complete (95%) rescue of melanoma cell growth by the addition of IL-8 100 ng/ml to curcumin 25 μ M pre-treated 1205Lu cells at 72% cell growth. Conclusion: Our in-vitro experiments along with literature survey suggested that IL-8 cytokine could be the molecule involved in the regulation of human melanoma cell growth and hence IL-8 could be considered as a target for melanoma treatment.

TITLE: 5-year outcomes in patients (pts) with ipilimumab (ipi)-refractory melanoma treated with pembrolizumab (pembro) in KEYNOTE-002

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ABSTRACT BODY:

Body: Checkpoint inhibitor therapy can result in durable responses with conversion of SD to PR or CR, and PR to CR. In KEYNOTE-002 (NCT01704287), pembro 2 mg/kg or 10 mg/kg improved PFS (HR 0.58 and 0.47; $P < 0.0001$ for both) with durable responses and manageable safety compared with chemo in pts with ipi-refractory melanoma. In this post hoc analysis, we assessed 5-year response and survival for the 361 pts who received pembro, combining both dose arms as there was no difference in dose efficacy. Response (RECIST v1.1; investigator review) was assessed at wk 12, every 6 wk until wk 48, then every 12 wk, and confirmed by subsequent scan. Survival was assessed every 12 wk. As of 30 May 2018, median follow-up was 58.5 mo. Median PFS was 4.2 mo (95% CI 3.3-5.6), and 60-mo PFS rate was 8%. Median OS was 14.0 mo (95% CI, 11.8-16.2), and 60-mo OS rate was 24%. 99 of 361 pts had CR (n=30) or PR (n=69) for ORR of 27.4% (95% CI, 22.9-32.3); 88 pts had SD. Median DOR was 54.7 mo (range, 1.9+ to 58.2+) and median time to response was 2.9 mo. Median DOR was not reached in pts with CR and was 54.7 mo in pts with PR. Median duration of SD was 7.0 mo. 8 (27%) pts with CR, 29 (41%) with PR and 63 (72%) with SD had subsequent PD. 55 of 357(15%) pts had grade 3-5 TRAEs (one grade 5 AE), and 25 (7%) discontinued due to a TRAE. In conclusion, responses to pembro are frequently durable with prolonged OS even in heavily pre-treated pts with ipi-refractory melanoma. Best responses evolved over time, with late conversions from SD to PR/CR and PR to CR.

TITLE: ALDH1 bio-activates nifuroxazide to eradicate ALDH^{High} melanoma-initiating cells

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ABSTRACT BODY:

Body: Majority of patients with metastatic melanoma despite the progress in targeted and immune based therapies, succumb to the disease. High ALDH1 (ALDH^{High}) activity marks distinct subpopulations in many cancers and ALDH^{High} cells are shown to be drug resistant with tumor-initiating (stem cell) potential. Melanoma is among the cancers with the highest levels of ALDH1 expression.

5-Nitrofurans are widely used anti-biotic and anti-parasitic drugs, that have recently emerged as having anti-cancer activity, although the mechanism of action in cancer is not well understood. Here, we discover that the 5-nitrofurans, nifuroxazide is selective for ALDH1 enzymes. Nifuroxazide is bioactivated by ALDH1 isoforms, whereby it both oxidizes ALDH1 and is converted to cytotoxic metabolites. Therefore, rather than merely inhibiting ALDH1 enzymes in living cancer cells nifuroxazide can eradicate ALDH1^{High} cancer cells in melanoma. We show that ALDH^{High} melanoma cells are sensitive to nifuroxazide, while ALDH1A3 loss-of-function mutations confer drug-resistance. In animal studies, nifuroxazide can inhibit melanoma tumor growth, and that nifuroxazide directly targets ALDH^{High} subpopulations in the tumour. Critically, we show that nifuroxazide induced loss of ALDH^{High} cells prevents subsequent tumor initiation potential. The clinical relevance of our discovery is underscored by our finding that ALDH1^{High} cells are enriched in melanomas post-vemurafenib treatment in matched patient samples, and that ALDH1 mRNA expression increases in melanomas from patients while on BRAF inhibitor plus MEK inhibitor combination therapy.

This work provides a novel and orthogonal therapeutic approach based on eliminating phenotypic subpopulations of cancer cells, and illustrates the larger issue of identifying and assessing the complex targets of clinically active drugs.

TITLE: Mutant STK19 promotes oncogenic NRAS-driven melanomagenesis

AUTHORS/INSTITUTIONS: C. Yin, B. Zhu, R. Cui, Boston University, Boston, Massachusetts, UNITED STATES|

ABSTRACT BODY:

Body: Activating mutations in NRAS account for 20-30% of melanoma, but despite decades of research and in contrast to BRAF, no effective anti-NRAS therapies have been forthcoming. Here we identify a previously uncharacterized serine/threonine kinase STK19 as a novel NRAS activator. STK19 phosphorylates NRAS to enhance its binding with its downstream effectors and consequently promotes NRAS-mediated melanocyte malignant transformation. A recurrent D89N substitution in STK19 whose alterations were identified in 25% of human melanomas with poor prognosis represents a gain-of-function mutation and interacts stronger with NRAS to enhance melanocyte transformation. STK19^{D89N} knockin leads to skin hyperpigmentation and promotes NRAS^{Q61R}-driven melanomagenesis in vivo. Finally, we developed ZT-12-037-01 (1a) as a specific STK19-targeted inhibitor. ZT-12-037-01 (1a) treatment effectively blocks oncogenic NRAS-driven melanocyte malignant transformation and melanoma growth in vitro and in vivo. Together, our findings provide a new and viable therapeutic strategy for melanomas harboring NRAS mutations.