TREATMENT WITH NEOADJUVANT + ADJUVANT DABRAFENIB AND TRAMETINIB (D+T) IS ASSOCIATED WITH IMPROVED RELAPSE-FREE SURVIVAL (RFS) VERSUS STANDARD OF CARE (SOC) THERAPY IN PATIENTS WITH HIGH-RISK RESECTABLE BRAF-MUTANT MELANOMA

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Body:
Treatment of stage IV melanoma pts has been revolutionized by new targeted and immune therapies. There is a strong rationale to evaluate their safety and efficacy in pts with earlier-stage disease. Current SOC for high-risk resectable melanoma (stage IIIB/C) is upfront surgery with or without adjuvant therapy. We hypothesized that neo+ adjuvant D+T treatment in this population would improve RFS over SOC.

We performed a randomized prospective clinical trial (NCT02231775) in pts with resectable stage IIIB/C or oligometastatic stage IV BRAF-mut melanoma. Pts were randomized in a 1:2 fashion to SOC (Arm A) or neo + adjuvant D+T (Arm B, 8 wks neo + 44 wks adjuvant D 150 mg BID + T 2 mg QD). Primary endpoint was RFS. Outcomes of an independent cohort of pts treated off-protocol with 8 wks of neo D+T (n=21) were also evaluated.

In the trial, 21 of the planned 84 patients were enrolled (arm A=7, arm B=14). Baseline characteristics were well balanced, and toxicity was manageable (grade 3 AEs in 27% pts on D+T). RECIST response rate after 8 wks of D+T was 77% and pCR rate at surgery was 58%. Treatment with neo + adjuvant D+T markedly improved RFS compared to SOC (HR 0.017, 95% CI <0.001 to 0.148, p<0.0001), leading to early stoppage of the trial. Immune analyses identified higher expression of T cell exhaustion markers (PD-1, Tim-3, and Lag-3) in tumors of pts that did not achieve a pCR (p<0.05). Similar RECIST (67%) and pCR (48%) rates were observed in the off-protocol cohort.

Treatment with neo + adjuvant D+T is well tolerated, achieves a high pCR rate, and improves RFS over SOC in pts with high-risk resectable melanoma. Correlative analyses offer insights into strategies to improve responses.
Uveal melanoma (UM) is the second most common form of melanoma and the most common ocular cancer. It is associated with visual morbidity with progression to metastasis occurring in ~50% of patients. Metastatic progression is invariably fatal, with 95% of cases metastasising to the liver. GNAQ and GNA11 are the only known driver oncogenes of UM, and are mutated in ~83% of cases, although neither are associated with prognosis. Rather, prognosis is determined by other factors such as chromosome rearrangements with monosomy of chromosome 3 being associated with poor prognosis. The tumour suppressor BAP1, which is located on chromosome 3, is mutated in ~40% of UM cases, ~80% of these co-occurring with chromosome 3 monosomy. While BAP1 loss-of-function (LOF) is associated with poor prognosis, the mechanism by which this drives UM and effects prognosis is still unclear.

To investigate, we generated isogenic cells lines for BAP1 LOF using CRISPR in human uveal melanoma cell lines. In vitro BAP1 LOF resulted in slower growth rates and changes in adherence properties. The formation of homologous recombination foci in response to DNA damage was also altered with BAP1 LOF and moderate sensitization to PARP inhibition was observed. In vivo BAP1 LOF resulted in more aggressive and faster growing tumours and most strikingly, favoured liver metastasis. This suggests BAP1 LOF may drive the liver tropism of metastatic UM observed in patients. BAP1 LOF results in numerous signalling and gene expression changes, which are being investigated to identify the basis of this tropism. A better understanding of how BAP1 LOF promotes metastatic disease and in particular the observed liver tropism, will aid understanding of metastatic UM and the identification of targets for therapeutic intervention.
RESULTS OF COLUMBUS PART 1: A PHASE 3 TRIAL OF ENCORAFENIB (ENCO) PLUS BINIMETINIB (BINI) VERSUS VEMURAFENIB (VEM) OR ENCO IN BRAF-MUTANT MELANOMA

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Body: BRAF inhibitor (BRAFi) monotherapy is effective in BRAF V600-mutant metastatic melanoma. Addition of a MEK inhibitor (MEKi) has been shown to improve survival, and attenuate some BRAFi-associated toxicities.

COLUMBUS is a 2-part, open-label phase 3 study of combination therapy with the BRAFi, ENCO, plus the MEKi, BINI, vs VEM or ENCO in unresectable/metastatic BRAFV600-mutant melanoma (first-line or progressed on/after prior immunotherapy). In Part 1, 577 pts [LDH high, M1c] were randomized 1:1:1 to ENCO 450 mg once daily (QD) + BINI 45 mg twice daily (BID) (COMBO450; n=192) [29%, 64%], VEM 960 mg BID (n=191) [27%, 65%], or ENCO 300 mg QD (n=194) [24%, 62%]. Primary and secondary endpoints were PFS per blinded central review (BCR) for COMBO450 vs VEM and vs ENCO, respectively. PFS HRs, per BCR, (95% CI) were 0.54 (0.41-0.71; P<0.001) for COMBO450 vs VEM and 0.75 (0.56-1.00; P=0.051) for COMBO450 vs ENCO. Median PFS was 14.9 (11-18.5), 7.3 (5.6-8.2), and 9.6 (7.5-14.8) mo for COMBO450, VEM, and ENCO, respectively. PFS HRs, per local assessment, were 0.49 (0.37-0.64; nominal P<0.001) for COMBO450 vs VEM and 0.68 (0.52-0.90; nominal P=0.006) for COMBO450 vs ENCO. Median PFS was 14.8 (10.4-18.4), 7.3 (5.7-8.5), and 9.2 (7.4-12.9) mo for COMBO450, VEM, and ENCO, respectively. AEs led to discontinuation in 12.5%, 16.7% and 14.1% of pts on COMBO450, VEM, and ENCO, respectively. The most common All Grade [%] AEs regardless of causality were nausea (41% COMBO450, 34 VEM, 39 ENCO), diarrhea (37, 34, 14), vomiting (30, 15, 27) fatigue (29, 31, 25) and arthralgia (26, 45, 44). The incidence [%] of (All Grades /Grade 3/4) photosensitivity/solar dermatitis and pyrexia were (5/<1 COMBO450, 30/1 VEM, 4/0 ENCO) and (18/4, 28/0, 15/1), respectively. COMBO450 significantly improved PFS vs VEM alone and was generally well tolerated.
**Title:** Development of a nuclear translocation inhibitor of ERK1/2 as an anti-cancer drug

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**Body:**

The ERK cascade plays a central role in the regulation of various cellular processes, including proliferation and differentiation. Therefore, its dysregulation leads to diseases such as cancer. One of the hallmarks of the cascade is the nuclear translocation of ERK1/2, which is important for the induction of proliferation. The nuclear translocation of ERK1/2 is mediated by the phosphorylation of its NTS, which allows its interaction with importin7 that carries ERK1/2 into the nucleus. We developed an NTS-derived peptide (EPE) that blocks the nuclear translocation of ERK1/2, by interfering with the formation of ERK1/2-importin7 complex, arresting active ERK1/2 in the cytoplasm. The EPE peptide significantly reduces the growth of several cancer lines, including Vemurafenib- and MEK inhibitor-resistant primary melanoma cells in culture and in vivo models. To develop more stable inhibitors of the nuclear translocation of ERK1/2, we performed high throughput screening of compounds with known pharmacological activity. We found 11 compounds that inhibited ERK1/2 nuclear translocation without affecting their activity. In addition, in silico approach resulted in the discovery of a new compound ZZZ, which blocks the nuclear translocation of ERK1/2. We found out that similarly to the EPE peptide, ZZZ interferes with the binding of ERK1/2-importin7 without inhibiting ERK1/2 activity. In addition, ZZZ inhibits proliferation/viability in several cancer cells, including BRAF-mutated melanoma. Further screening of 23 chemical analogues of ZZZ, led to the discovery of a new compound, that we termed C17, with improved efficacy at lower concentration and low toxicity. Taken together, our results indicate that the mechanism of the nuclear translocation of ERK1/2 serves as potential target for cancer therapy.
TITLE: Pooled analysis of safety with extended 3-year follow-up across combination dabrafenib and trametinib (D+T) phase 3 trials

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Body: Significantly improved outcomes with D+T vs BRAF inhibitor (BRAFi) monotherapy have been demonstrated in phase 3 BRAF V600E/K–mutant melanoma trials (COMBI-d [NCT01584648]; COMBI-v [NCT01597908]), supporting use of D+T as a treatment for this disease. Additionally, long-term survival and sustained tolerability have been observed in a significant number of patients (pts) with D+T in these trials. As pts achieve long-term benefits, understanding the safety profile of these agents with extended follow-up is important for optimizing pt management. A prior pooled analysis of safety data in D+T cohorts across registration trials (median follow-up, 20 mo) showed that the highest adverse event (AE) rates were observed within 6 mo of D+T initiation and that, although common with D+T, occurrence of pyrexia during treatment did not predict response or progression. This is a 3-y pooled safety analysis of D+T pts across COMBI-d (n=209; cutoff, Feb2016) and COMBI-v (n=350; cutoff, Jul2016). AEs were graded per NCI CTCAE v4.0 and assessed by time. Consistent with previous reports for D+T, the most frequent AEs across the pooled data set (N=559) with extended follow-up were pyrexia (58%), nausea (36%), chills (33%), diarrhea (33%), fatigue (33%), vomiting (29%), and hypertension (29%). In pts receiving D+T for ≥36 mo (n=50; 9%), AE incidence generally peaked within 6 mo of treatment initiation and declined thereafter (eg, pyrexia rate was 60% from 0-6 mo, 24% from 6-12 mo, 32% from 12-18 mo, 22% from 18-24 mo, 24% from 24-32 mo, and 12% from 32-36 mo). These results confirm that long-term tolerability of D+T is achievable in pts with BRAF-mutant melanoma. Additional analyses of outcomes in pts who had D+T dose reductions or discontinuation, as well as detailed recurrent pyrexia management strategies, will be presented.
TITLE: IL-6 as a potential plasma biomarker of response to combined ipilimumab and anti-PD-1 therapy in melanoma

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Body: Immune checkpoint inhibitors ipilimumab and the anti-PD-1 agents pembrolizumab and nivolumab have significantly improved survival rates of metastatic melanoma patients. Anti-tumor activity is increased by combining anti-PD1 with the anti-CTLA-4 drug ipilimumab, but toxicity is substantially greater and around one-third of patients still show minimal tumor response. We explored circulating protein profiles in search of potential plasma biomarkers of response in patients with metastatic melanoma treated with anti-PD1/anti-CTLA-4 combinations.

Melanoma patients treated with anti-PD-1 and anti-CTLA-4 had plasma samples drawn before (baseline) and early during treatment (within 3 weeks of treatment initiation). 12 good responders (CR or PR by immune related RECIST criteria) and 12 poor responders (PD or SD) were retrospectively identified for initial comparison. Plasma samples were evaluated for expression of ~1300 distinct proteins using a novel proteomics assay (SomaLogic).

Proteomic analysis revealed no substantial differences in the baseline plasma of good responders vs. poor responders. In contrast, expression of several proteins including IL-6, the tyrosine-protein kinases Lyn and Fyn were significantly elevated in early during therapy plasma of non-responders compared to responders. In preliminary functional studies, IL-6 stimulated proliferation and MHC Class II expression in 2 of the 3 melanoma cell lines tested. The effects of IL-6 on the host-tumor interaction are likely to be protean, but may include a propensity to confer a growth advantage in melanoma cells. These findings indicate IL-6 as a potential marker of poor response to combined immunotherapy in vivo and are being further analyzed in an independent validation set.
Title: Three-year pooled analysis of baseline and postbaseline factors associated with clinical benefit with combination dabrafenib and trametinib (D+T) across phase 3 trials

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Body: Front-line use of D+T in BRAF V600–mutant melanoma has been supported by phase 3 studies showing significantly improved efficacy with D+T vs BRAFi monotherapy and long-term survival of many patients (pts) treated with D+T (COMBI-d [NCT01584648]; COMBI-v [NCT01597908]). With multiple therapy options, understanding clinical factors that predict long-term benefit with D+T is key for optimizing pt management. A prior pooled analysis of factors that predict outcomes in D+T pts across registration trials (median follow-up [f/u], 20 mo) identified baseline lactate dehydrogenase (LDH) and number of organ sites with metastasis as the most influential factors for progression-free survival (PFS) and overall survival (OS). Longer-term f/u analyses are needed to confirm which pts treated with D+T can achieve maximum benefit. This 3-y pooled analysis of D+T pts across COMBI-d (n=211; cutoff, Feb2016) and COMBI-v (n=352; cutoff, Jul2016) assessed factors identified a priori (age, BRAF status, stage, ECOG performance status, sex, LDH, number of organ sites with metastasis, prior adjuvant immunotherapy, sum of target lesion diameter, response) for association with PFS and OS using univariate, multivariate, and regression tree analyses. In the pooled data set (N=563), 3-y PFS (24%) and OS (44%) were consistent with individual trials. As previously identified, baseline LDH and number of organ sites with metastasis remained strongly associated with PFS and OS. Additionally, with extended 3-y f/u, baseline lesion burden was also identified as a predictor for PFS. Pts with normal LDH, <3 organ sites with metastasis, and baseline lesion diameter <66 mm (n=184) had the longest PFS (3-y, 41%) and OS (3-y, 70%). Additional analyses of predictive factors among pts with defined time points of therapy duration will be presented.
Body: Cutaneous, acral and mucosal subtypes of melanoma (n=183) were evaluated by high coverage, whole-genome sequencing. The heavily mutated landscape of coding and non-coding mutations in cutaneous melanoma displayed signatures of known and novel ultraviolet radiation mutagenesis. In marked contrast, acral and mucosal melanomas were dominated by structural changes and mutation signatures of unknown aetiology, not previously identified in melanoma. The number of genes affected by recurrent mutations disrupting non-coding sequences was similar to that affected by likely driver mutations of coding sequences. Mutations affecting the TERT promoter were the most frequent of all, however neither they nor ATRX mutations, associated with the alternative telomere lengthening mechanism, were correlated with greater telomere length. Most melanomas had potentially actionable mutations, and most of these were in components of the mitogen-activated protein kinase and phosphoinositol kinase pathways.
Body: The phase 1b BRIM7 study identified the dose, schedule, and preliminary efficacy of C+V in patients (pts) with advanced BRAFV600-mutated melanoma. The phase 3 coBRIM study demonstrated that C+V significantly improved progression-free survival (PFS) and overall survival (OS) vs placebo (P)+V in this population. Long-term safety and efficacy data for C+V are presented from both studies. For coBRIM, at the data cutoff of June 2016, with median follow-up of 21.2 (1.4-39.5) months for C+V and 16.6 (0.5-38.6) months for P+V, median OS was 22.5 months for C+V vs 17.4 months for P+V. Landmark OS rates at 1, 2, and 3 years were 74.5%, 49.1%, and 37.4%, respectively, for C+V and 63.8%, 38.6%, and 31.1% for P+V. BRIM7 evaluated C+V in BRAF inhibitor (BRAFi)-naive pts and in pts who had progressed on prior vemurafenib treatment (Vem-PD). At a data cutoff of April 2016 with median follow-up of 25.8 months, median PFS for BRAFi-naive pts was 13.8 months and median response duration was 14.3 months. For Vem-PD pts with median follow-up of 8.3 months, median PFS was 2.8 months and median response duration was 6.8 months. Median OS was 31.2 months in BRAFi-naive pts, with landmark OS rates at 2, 3, and 4 years of 63.9%, 39.2%, and 35.9%. In Vem-PD pts, median OS was 8.5 months, with landmark OS rates at 2 and 3 years of 17.5% and 12.5% but 4 years not yet reached. The safety profile for both studies was similar to that reported previously. Discontinuation rates due to adverse events across both studies were <20%. In coBRIM, 54.5% of C+V and 57.7% of P+V pts who discontinued both drugs had ≥1 subsequent anticancer treatment. These data from extended follow-up of the 2 studies confirm the clinical benefit of continued C+V in patients with advanced BRAFV600-mutated melanoma.
TITLE: Phase 1b/2, open-label, multicenter, dose escalation and expansion trial of intratumoral SD 101 in combination with pembrolizumab in patients with metastatic melanoma

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Body: SD-101 is a synthetic Class C CpG-ODN that stimulates plasmacytoid dendritic cells. Pembrolizumab is a PD-1 inhibitor that has been approved for the treatment of metastatic melanoma. This study, DV3-MEL-01 trial (NCT02521870), assesses the safety and preliminary efficacy of SD-101 (injected intratumorally wkly x 4 then q3 wkly x 3) + pembrolizumab (200 mg IV q3wk) in unresectable stage III-IV melanoma. Here we report data from 2 cohorts at 2 mg (5 pts) and 4 mg (5 pts), of whom 50% are pts who previously progressed on anti-PD-1. Tumor responses are assessed using RECIST v1.1. Samples for biomarkers assessments pre- and post-treatment were taken. There have been no DLTs to date. Treatment-related adverse events (AE) were observed in 2 pts in the 4 mg cohort, 1 injection site pain and a 2nd pt had an SAE of cellulitis resulting in discontinuation associated with pain in extremity/erythema. Two unrelated SAEs of squamous cell ca and sepsis were observed in the 2 mg cohort. The safety profile of SD-101 was consistent with prior reports with most commonly seen AEs of grade 1-2 flu-like symptoms and injection-site reactions. No increased toxicity of the combination of drugs has been observed. With follow-up from 64 to 190 days, the best overall response observed were for anti-PD-1 naïve CR 20%, PR 60%, SD 0%, PD 20% and for anti-PD-1 progressor CR 0%, PR 0%, SD 60%, PD 40%. Additionally, mechanistic insight into therapeutic activity obtained through examination of tumor biopsies by NanoString RNA expression profiling provides evidence of an increase in immune cell infiltrate into the tumor microenvironment in injected lesions that trends with objective tumor responses. These results suggest that the combination of SD-101+pembrolizumab shows early signs of additive efficacy in metastatic melanoma.
TITLE: Combined inhibition of MDM2 and CDK4/6: a promising new treatment approach for NRAS-mutant melanoma

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Body: Up to 20% of melanomas are characterized by NRAS driver mutations, but effective therapy targeting NRAS is not available. Our preliminary in vitro studies showed that combining MDM2 and CDK4/6 inhibitors has a greater effect on proliferation and viability of NRAS-mutant melanoma cells than using either drug alone. We hypothesized that combined MDM2 and CDK4/6 inhibitors would decrease growth and proliferation of NRAS-mutant melanomas to a greater extent than single agent treatments in vivo. Our secondary aim was to identify genetic and protein markers of therapeutic response. Post-operative NRAS-mutant patient melanomas were subjected to targeted next generation sequencing and implanted into nude mice to generate patient-derived xenografts (PDXs). Mice were treated with vehicle, MDM2 inhibitor (50 mg/kg), CDK4/6 inhibitor (100 mg/kg), or combined inhibitors. Tumor growth rates and weights were compared with a nested mixed-effects regression model and the Kruskal-Wallis test, respectively. To date, we have tested five distinct NRAS-mutant melanoma PDXs. We observed significant inhibition of tumor growth with combined therapy in 80% of the tumors. In 75% of responder PDXs, combined CDK4/6 and MDM2 inhibition was more effective than single agent treatments. Tumor sequencing did not reveal known pathologic TP53, CDKN2A, Rb1, or BRAF mutations. Upregulation of p21 and p53, activation of Rb, downregulation of p-Histone-H3, and decreased Ki-67 expression was found in tumors after combined therapy. Our results demonstrate that combined MDM2 and CDK4/6 inhibitors are more effective than either therapy alone for a subset of NRAS-mutant human melanomas. Potential pharmacodynamic markers of response to combination therapy include Rb and p21. Our findings provide a rationale for further development of combined MDM2 and CDK4/6 inhibitor therapy, informing future clinical trials.
**Title**: Bevacizumab as a steroid-sparing agent in the immunotherapeutic treatment of melanoma brain metastases

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**Body**: Immune checkpoint blockade has revolutionized metastatic melanoma treatment but immunosuppression needs to be minimized to maximize response. This presents a dilemma in patients with brain metastases requiring immunosuppressive corticosteroids to control symptoms. Here we report use in such patients of bevacizumab (BEV), a VEGF-inhibitor that also augments anti-tumor immune responses, as a steroid-sparing agent.

Medical records and imaging were retrospectively analyzed for 11 melanoma patients with brain metastases who received BEV (5-7.5mg/kg Q2-3W, median cycles 4, range 1-9) between 2012 and 2016. All patients (3 BRAF wild type, 8 BRAF mutant resistant to BRAF/MEK inhibitors) had progressive intracranial disease with peritumoral edema after prior resection, stereotactic radiosurgery and/or whole brain radiotherapy. 8 patients required dexamethasone (median 8mg/day) prior to BEV and half weaned to ≤2mg only 4 weeks after BEV; steroids were successfully avoided in the remainder. 2 patients did not receive immunotherapy (rapid progressive disease, patient choice) and were excluded from further analysis. Of 9 evaluable patients, 7 had improved edema and symptoms. 9 patients had hemorrhagic metastases prior to BEV; new hemorrhage occurred in 2 after BEV (G1, G5). Other adverse events: G3 hypertension and G5 gastrointestinal bleeding from bowel metastases. Of 9 patients who received anti-CTLA4 or anti-PD1 immunotherapy, intracranial disease control rate was 44%. 4 patients survived >6 months, including 1 who is disease-free despite being hemiplegic from edematous brain metastases upon commencing BEV >3 years ago.

In this case series of 11 melanoma patients with brain metastases, BEV was well-tolerated, associated with improved symptoms and reduced peritumoral edema despite weaning steroids, and facilitated treatment with immunotherapy that provided durable survival.
A Phase 1 study of IMCgp100, a soluble HLA-A2 restricted gp100-specific T cell receptor-CD3 therapeutic with solid tumor activity in patients with advanced uveal melanoma

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Body: T cell-based immunotherapy has proven successful for multiple tumor types; however, efficacy in uveal melanoma (UM) remains elusive. IMCgp100 is a bispecific biologic capable of redirecting T cells against the tumor-associated antigen gp100, which is highly expressed in UM. In the preclinical setting, IMCgp100 has been shown to re-direct T cells to kill gp100+ UM cells with high potency. The first-in-human study with IMCgp100 completed enrolment (n=84). Patients with both cutaneous and UM were treated in dose escalation and in the RP2D expansion cohort. Dose limiting toxicity was grade 3 or 4 hypotension associated with a profound trafficking of CD3+ T lymphocytes into skin and tumor, as evidenced on biopsies. Frequent toxicities (any grade) include rash (69%), pruritus (64%), pyrexia (52%), periorbital edema (46%), fatigue (42%) and nausea (39%). Toxicities of grade ≥3 were observed infrequently and generally after doses 1 and 2, including rash (15%), lymphopenia (14%), and hypotension (9%). The dosing regimen includes an intra-patient escalation to mitigate the risk of cytokine release with the first 2-3 doses. In this study, 15 patients with UM were treated at ≥270ng/kg QW and are evaluable for efficacy. At baseline, patients presented with elevated LDH (67%) and hepatic metastases (87%). Among patients with UM, three (20%) patients had partial responses in both liver and extra-hepatic disease (one unconfirmed, ongoing), and seven (47%) patients had stable disease. The disease control rate at 16 weeks is 53% and at 24 weeks is 40%. IMCgp100 has a favorable safety profile and preliminary Phase 1 results suggest activity in UM. An ongoing Phase 1 in UM will identify the escalated dose that can be administered in the intra-patient escalation regimen and the pivotal study in UM is planned.
Epigenetic agents have drawn great attention as anti-cancer therapies, with several HDAC inhibitors approved for a subset of hematologic malignancies. One of the biggest challenges in targeting epigenetic mechanisms of tumorigenesis is the wide spectrum of effects which restrict the therapeutic window for these compounds. We have developed a series of potent small molecule inhibitors with specificity towards the CoREST epigenetic corepressor complex through a dual-action mechanism targeting LSD1 and HDAC1. These compounds show a unique profile of pharmacologic action with an improved therapeutic window in a variety of cell types. Screening of tumor cell lines for growth inhibitory effects revealed variable efficacy in a broad spectrum of cancers with the most consistent and potent effects seen in human melanomas. Melanoma cell lines were found to be uniformly inhibited by the most potent and specific of these compounds, Corin 2; however, primary human melanocytes showed no sensitivity to these agents. Transcriptomic analysis revealed that corin2 was a more potent inducer of tumor suppressor genes compared to the parent HDAC and LSD1 compounds. Genetic knockdown of CoREST or LSD1 in cancer cell lines abolished the differences in potency of Corin2 vs. the parent HDAC inhibitor, Entinostat, suggesting that Corin2’s favorable pharmacologic effects rely on an intact CoREST complex. Corin2 was also effective in slowing tumor growth in a melanoma mouse xenograft model. These dual action inhibitors demonstrate a novel, potent, and specific therapeutic approach to targeting epigenetic pathways in human melanomas which may lead to improved therapeutic benefits in patients with advanced disease.
TITLE: Induction of Telomere Dysfunction Prolongs Control of Therapy-Resistant Melanoma

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Body: Immune checkpoint blockade inhibitors and mitogen-activated protein kinase (MAPK) inhibitors have emerged as first-line therapies for patients with advanced melanomas. Despite unprecedented initial successes, the majority of patients ultimately relapse and have limited options of second-line therapies. For patients who fail multiple therapies, there is an unmet and urgent need to prolong disease control. Telomerase (TERT) promoter mutations are highly prevalent in melanoma cell lines, therapy-naïve and -resistant tumors. Telomere transcriptional signatures are enriched not only in a subset of therapy-naïve melanomas associated with worse overall survival, but also in BRAF-mutant intrinsically resistant melanoma cells that evade MAPK inhibitors (MAPKi). Telomere transcriptional gene signatures are also enriched in a subset of post-treatment tumor biopsies derived from patients who have disease progression on MAPKi or the immune checkpoint inhibitor, ipilimumab that targets CTLA-4. We demonstrate the efficacy of a telomerase-directed nucleoside, 6-thio-2'-deoxyguanosine (6-thio-dG) in BRAF-mutant melanoma cells that results in telomere dysfunction and cell death. Further, 6-thio-dG significantly inhibits tumor growth of primary tumor biopsy cultures derived from patients who had disease progression on multiple therapies including ipilimumab and pembrolizumab that targets the programmed cell death 1 (PD-1) receptor. Thus, 6-thio-dG may be a viable approach to overcoming cancer therapy resistance.
The role of DNA repair signaling in the survival and progression of melanoma

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Body: Despite recent improvements in the treatment of melanoma, achieving long-term responses in most patients remains a challenge. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism of tumour cell survival. Thus, inhibition of DNA repair may sensitise tumour cells to cancer therapies. We initially analysed whole exome sequence data from 367 metastatic melanoma samples obtained from The Cancer Genome Atlas (TCGA) project, to identify the relative frequency of alterations affecting DNA damage response (DDR) pathways. Our analyses revealed that DDR pathways are not targeted for alterations in melanoma and, thus, we propose that DNA damage signaling may confer a survival advantage to melanoma cells. A more exhaustive analysis of different DDR pathways showed that the NRAS-mutant melanomas expressed a higher percentage of alterations affecting the NER (nucleotide excision repair) and p53 pathways, compared to BRAF-mutant melanomas. In contrast, the NHEJ (non-homologous end joining) pathway did not display an enrichment of mutations suggesting that NRAS or BRAF mutant melanoma may rely on the NHEJ pathway for repair of genotoxic stress. To confirm the above analysis, we are evaluating the activity of individual DNA repair pathways in a panel of melanoma cell lines, using a host cell reactivation assay that enables real-time live cell quantitative analysis. Identification of impaired and functioning DNA repair pathways in melanoma will provide insights into the role of genomic instability in disease progression, and may also lead to the development of novel combination therapies.
Title: BRD9 (Bromodomain containing protein 9) in Melanogenesis and Melanoma Proliferation

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Body: SWI/SNF chromatin remodeling complexes regulate the expression of genes important for melanogenesis and melanoma proliferation. Heterogeneous SWI/SNF complexes that contain either BRG1 or BRM as the catalytic subunit and an assortment of associated factors (BAFs) have been identified. BRG1 and BRM as well as some BAFs have bromodomains (BrD) which bind to acetylated lysine residues in histone tails. Little is known about the role of bromodomain proteins in regulating SWI/SNF function. Small molecules that specifically inhibit the association of these BrD-containing proteins with chromatin can be used as tools to interrogate Brd function and may have therapeutic potential. I-BRD9 is a chemical inhibitor specific for BRD9, a newly identified BrD-containing component of SWI/SNF complexes that have BRG1 as the catalytic subunit. We found that BRD9 is highly expressed in melanocytes and melanoma cell lines. Co-immunoprecipitation studies indicated that BRD9 and BRG1 physically interact in melanoma cells. To test the hypothesis that BRD9 has a function in melanogenesis and melanoma proliferation, we treated melanocyte precursors with I-BRD9. I-BRD9 inhibited melanin synthesis and expression of genes that regulate melanocyte function. Decreased expression of genes that regulate melanin synthesis was associated with altered chromatin structure at regulatory sites. Depletion of BRD9 by siRNA had similar effects on gene expression as treatment with I-BRD9. Furthermore, treatment of melanoma cells with I-BRD9 compromised proliferation and colony survival. In combination, our data indicate that Brd9 has an important role in regulating melanogenesis and melanoma proliferation and that chemical inhibition may be useful for treating melasma and melanoma.
Chemokines are a family of low molecular weight proteins secreted by cells. Many of them have been detected in melanoma cells, and play critical roles in either tumor progression, or tumor associated immune responses. We and others have previously shown that Notch signaling is involved in melanoma growth and progression. Interestingly, we find that Notch signaling modulates the expression of chemokines in melanoma. In our chemokine array data, the expression and/or secretion of IL8, CCL5 (RANTES) and IL6 were down regulated by Notch1 knock down in melanoma cell lines. Interestingly, SNAP23, which is involved in cell exocytosis processes, was also found inhibited by Notch1 knock down. shRNA-mediated inhibition of SNAP23 resulted in reduced secretion of both CCL5 and IL8 in melanoma cells. We therefore concluded that Notch1 modulates chemokine levels by regulating their expression, and by modulating their secretion through SNAP23.

IL6, IL8 and CCL5 have been previously implicated in Myeloid Derived Suppressor Cells expansion and recruitment. In melanoma, circulating MDSCs have a negative impact on survival and inversely correlate with the presence of functional antigen-specific T cells. Also, melanoma patients with higher circulating MDSCs respond less to immunotherapies. Interestingly, we find that inhibition of Notch1 by shRNAs reduces infiltrating MDSCs while, at the same time, leads to an increase in CD8+ T cells in the melanoma tumor microenvironment.

Based on these data, we hypothesize that Notch1 signaling plays a critical role in tumor associated immunosuppression by modulating the recruitment of MDSCs to the expense of cytotoxic T cells likely via the expression and secretion of key chemokines. The goal is to further establish the mechanisms underlying Notch1 mediated immunosuppressive function and to determine whether Notch1 inhibition would improve the efficacy of checkpoint immunotherapies.