Adenosine Signaling Is Prognostic for Cancer Outcome and Has Predictive Utility for Immunotherapeutic Response

Ben Sidders[1], Pei Zhang[1], Kelly Goodwin[2], Greg O'Connor[3], Deanna L. Russell[3], Alexandra Borodovsky[2], Joshua Armenia[1], Robert McEwen[1], Bolan Linghu[3], Johanna C. Bendell[4], Todd M. Bauer[4], Manish R. Patel[5], Gerald S. Falchook[6], Melinda Merchant[7], Gayle Pouliot[7], J. Carl Barrett[3], Jonathan R. Dry[3], Rich Woessner[2], and Kris Sachsenmeier[3]

ABSTRACT

Purpose: There are several agents in early clinical trials targeting components of the adenosine pathway including A2AR and CD73. The identification of cancers with a significant adenosine drive is critical to understand the potential for these molecules. However, it is challenging to measure tumor adenosine levels at scale, thus novel, clinically tractable biomarkers are needed.

Experimental Design: We generated a gene expression signature for the adenosine signaling using regulatory networks derived from the literature and validated this in patients. We applied the signature to large cohorts of disease from The Cancer Genome Atlas (TCGA) and cohorts of immune checkpoint inhibitor–treated patients.

Results: The signature captures baseline adenosine levels *in vivo* ($r^2 = 0.92$, $P = 0.018$), is reduced after small-molecule inhibition of A2AR in mice ($r^2 = -0.62$, $P = 0.001$) and humans (reduction in 5 of 7 patients, 70%), and is abrogated after A2AR knockout. Analysis of TCGA confirms a negative association between adenosine and overall survival (OS, HR = 0.6, $P < 2.2e^{-16}$) as well as progression-free survival (PFS, HR = 0.77, $P = 0.0000006$). Further, adenosine signaling is associated with reduced OS (HR = 0.47, $P < 2.2e^{-16}$) and PFS (HR = 0.65, $P = 0.0000002$) in $CD8^+$ T-cell–infiltrated tumors. Mutation of TGFβ superfamily members is associated with enhanced adenosine signaling and worse OS (HR = 0.43, $P < 2.2e^{-16}$). Finally, adenosine signaling is associated with reduced efficacy of anti-PD1 therapy in published cohorts (HR = 0.29, $P = 0.00012$).

Conclusions: These data support the adenosine pathway as a mediator of a successful antitumor immune response, demonstrate the prognostic potential of the signature for immunotherapy, and inform patient selection strategies for adenosine pathway modulators currently in development.

Introduction

The role of the immune system in controlling cancer is widely recognized (1). Therapeutically, this is evidenced by a number of recent drug approvals for immunotherapy agents that enhance endogenous antitumor immunity (reviewed by ref. 2) or target tumors directly (reviewed by ref. 3). Responses to immunotherapies are distinct from those seen from other targeted therapies in at least two respects. First, these responses are being observed in cancer indications of previously unmet need such as melanoma (4), lung adenocarcinoma (5), and hematologic malignancies (6). Second, the duration of response to immunotherapy appears to persist for longer in certain settings than those observed with targeted therapies (reviewed by ref. 7).

Despite the clinical success of immunotherapy, there are important questions regarding the initial or eventual failure to control disease, and the value of targeted versus broader approaches to tumor immunity. Current immunotherapies target specific molecules within the immune system, such as the checkpoint proteins PD1 and PDL1, and show responses in only a subset of patients with cancer in any given indication. Total mutational burden (TMB; ref. 8) and PDL1 protein (9) levels have been shown to correlate with immunotherapy response. However, only 30% of the responders are positive based upon these measures (10), suggesting that a more widespread response may be achieved by taking a broader approach; for example, by targeting both innate and adaptive antitumor immunity.

One example of a factor with broad immunosuppressive effects is the adenosine signaling axis (11), which has been shown to suppress natural killer (NK) and $CD8^+$ T-cell cytolytic activity while enhancing suppressive macrophage and dendritic cell polarization as well as Treg and myeloid-derived suppressor cell (MDSC) proliferation (12). Beginning with landmark research by Ohta and colleagues (13), a series of preclinical studies (14–18) have been reported and clinical trials (19–21) initiated targeting adenosine signaling. In addition, preclinical evidence supports a role for adenosine axis antagonists in chimeric antigen receptor T-cell therapy (22), adoptive cell therapy (13), and cancer vaccines (23). Thus, targeting the adenosine axis may block a broadly relevant immunosuppressive pathway in cancer (24). Fong and colleagues have demonstrated tumor regressions in 24% of patients with renal cell cancer treated with an A2AR antagonist (25).

We sought to characterize the pan-cancer role of adenosine in human tumors, to test the hypothesis that adenosine signaling has a

[1]Translational Medicine, Research and Early Development, Oncology R&D, AstraZeneca, Cambridge, United Kingdom. [2]Discovery, Research and Early Development, Oncology R&D, AstraZeneca, Boston, Massachusetts. [3]Translational Medicine, Research and Early Development, Oncology R&D, AstraZeneca, Boston, Massachusetts. [4]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, Tennessee. [5]Sarah Cannon Research Institute/Florida Cancer Specialists, Sarasota, Florida. [6]Sarah Cannon Research Institute/HealthONE, Denver, Colorado. [7]Early Clinical Development, Research and Early Development, Oncology R&D, AstraZeneca, Boston, Massachusetts.

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Corresponding Author: Ben Sidders, Bioinformatics and Data Science, Translational Medicine, Oncology R&D, AstraZeneca, 1 Francis Crick Avenue, Cambridge CD2 0AA, United Kingdom. Phone: 44-7464-982513; E-mail: benjamin.sidders@astrazeneca.com

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Translational Relevance

There are several agents in early clinical trials targeting components of the adenosine pathway including A2AR and CD73. The identification of cancers with a significant adenosine drive is critical to identify patients who might benefit from these therapies. However, it is challenging to measure tumor adenosine levels in a high-throughput manner. Thus novel, clinically tractable biomarkers are needed. We generate and validate a gene expression signature that recapitulates adenosine signaling activity. We demonstrate that adenosine signaling is associated with survival across tumors of all types and within specific indications, and that baseline adenosine signaling scores predict response to immune checkpoint therapies. The signature reported here therefore has the potential to inform patient selection strategies for adenosine pathway modulators and existing immunotherapies.

negative prognostic link within human tumors, and to identify segments of disease where this might be most pronounced. Ideally, a biomarker to address this question would be derived independently of any particular adenosine axis–targeting agent, be quantitatively measurable across various tumor indications, and test the hypothesis using existing public molecular databases. Here, we report the discovery and characterization of a gene expression signature that meets these criteria and which we use to define the landscape of adenosine signaling in human cancer with implications for existing immune checkpoint therapies and adenosine targeting agents in development.

Materials and Methods

Signature generation and scoring

To define a network of regulatory interactions for the A2AR receptor, we used two complementary datasets. Natural Language Processing (NLP) of abstracts and open-access full text from Medline and PubMed Central was performed by Biorelate Ltd (https://www.biorelate.com/) to broadly sweep as much of the literature as possible. In contrast, knowledge derived purely from manual curation in the Ingenuity Pathway Analysis tool database (Qiagen) was used to provide a deeper mining of full text articles from a smaller set of high-impact journals targeted by that resource.

Biorelate defines a causal (regulatory) interaction as a relationship between two entities (genes or proteins) where the subject (cause) entity has a directed edge with an object (theme) entity. Gene entity terms and their relationships from their in-house dictionaries were matched through their machine learning–named entity-recognition software, now incorporated within Biorelate Galactic AI. Protein entities from human, mouse, and rat were retained under the expectation that human data would be the most relevant, while mouse and rat would capture the majority of animal models used in biomedical research. Causal interactions were then collapsed such that all events containing the same pair of entities and the same interaction type were grouped. These groups were assigned a confidence score that was used to rank select events for manual verification.

We then filter the combined set of regulatory relationships to identify genes that are downstream of A2AR (154 genes; 136 from manual curation and 18 from NLP, with 13 detected in both), upregulated by A2AR (90 genes; 78 from manual curation and 12 from NLP), robustly expressed in human tumors, defined as having a median expression greater than the median expression of all genes (74 genes; 66 from manual curation and 8 from NLP), and, finally, by their presence on the Nanostring PanCancer Immune Profiling expression panel (14 genes; 10 from manual curation and 4 from NLP). This last step ensures that our signature retains maximum clinical utility given that the Nanostring panel is widely used to profile formalin-fixed, paraffin-embedded (FFPE) samples from clinical trials where whole transcriptome profiling is often unavailable. The 14 genes that meet these criteria and form the signature are: *PPARG*, *CYBB*, *COL3A1*, *FOXP3*, *LAG3*, *APP*, *CD81*, *GPI*, *PTGS2*, *CASP1*, *FOS*, *MAPK1*, *MAPK3*, and *CREB1*.

We score transcriptome data with the signature using Gene Set Variation Analysis [GSVA (26)]. This method is robust to outlying genes expressed at different orders of magnitude and generates scores amenable to downstream statistical interpretation. We observe a strong linear correlation between signatures representing immune processes/features and tumor purity in The Cancer Genome Atlas (TCGA; see Supplementary Fig. S1). To account for this bias, we adjust the signature scores for tumor purity by fitting a linear regression derived from all samples in TCGA versus tumor purity and then applying the following correction:

$$\text{Corrected score} = \text{uncorrected score} - (\text{intercept} + \text{slope} \times \text{purity})$$

Analysis of public datasets

Exome sequencing data from TCGA were processed as described in ref. (27). TCGA RNA sequencing (RNA-seq) data were described in ref. (28), and associated clinical data were taken from ref. (29). Copy-number variants made with GISTIC version 2.0.22 were obtained from the TCGA Firehose. Microsatellite instability (MSI) subtype information was obtained from ref. (30). Tumor purity data were obtained from ref. (31).

RNA-seq data from *ADORA2A* knockout NK cell lines generated in ref. (32) were obtained from the European Nucleotide Archive (PRJEB22631). Reads were aligned to the mouse genome (mm10) using HISAT2 (33), and expression levels were quantified using Salmon (34).

Published cohorts of immunotherapy-treated subjects with preprocessed gene expression profiles were obtained from refs. (35, 36) and scored with GSVA. The anti–CTLA-4 dataset (36) was generated with a custom nanostring panel that contained only 6 (*CASP1*, *CD81*, *CYBB*, *LAG3*, *PARG*, and *PTGS2*) of the 14 genes from our signature.

Survival analysis

Survival analyses were performed using the Cox proportional hazards regression model as implemented in the Survival package from R (37). For the analysis presented in **Fig. 2A** and **B**, tumors were split into high (>75th), medium (25–75th), and low (<25th) based on quartiles. In all other survival analyses, adenosine signature scores were split on 0 with >0 high and <0 low.

Immune cell–type infiltrate scoring

Immune cell infiltrates were determined with a support vector regression (SVR) approach based on CIBERSORT (38) to define relative immune cell abundance. To study the association of adenosine with $CD8^+$ T-cell infiltration, we consider CD8 high tumors to be greater than the median of *CD8A* expression across all samples. All other cell or cell-state signatures were scored using GSVA. NK-cell exhaustion was determined using expression of *KIR3DL1*, *KIR3DL2*, *IL2RA*, *IL15RA*, *HAVCR2*, and *EOMES*. Cytotoxicity was determined

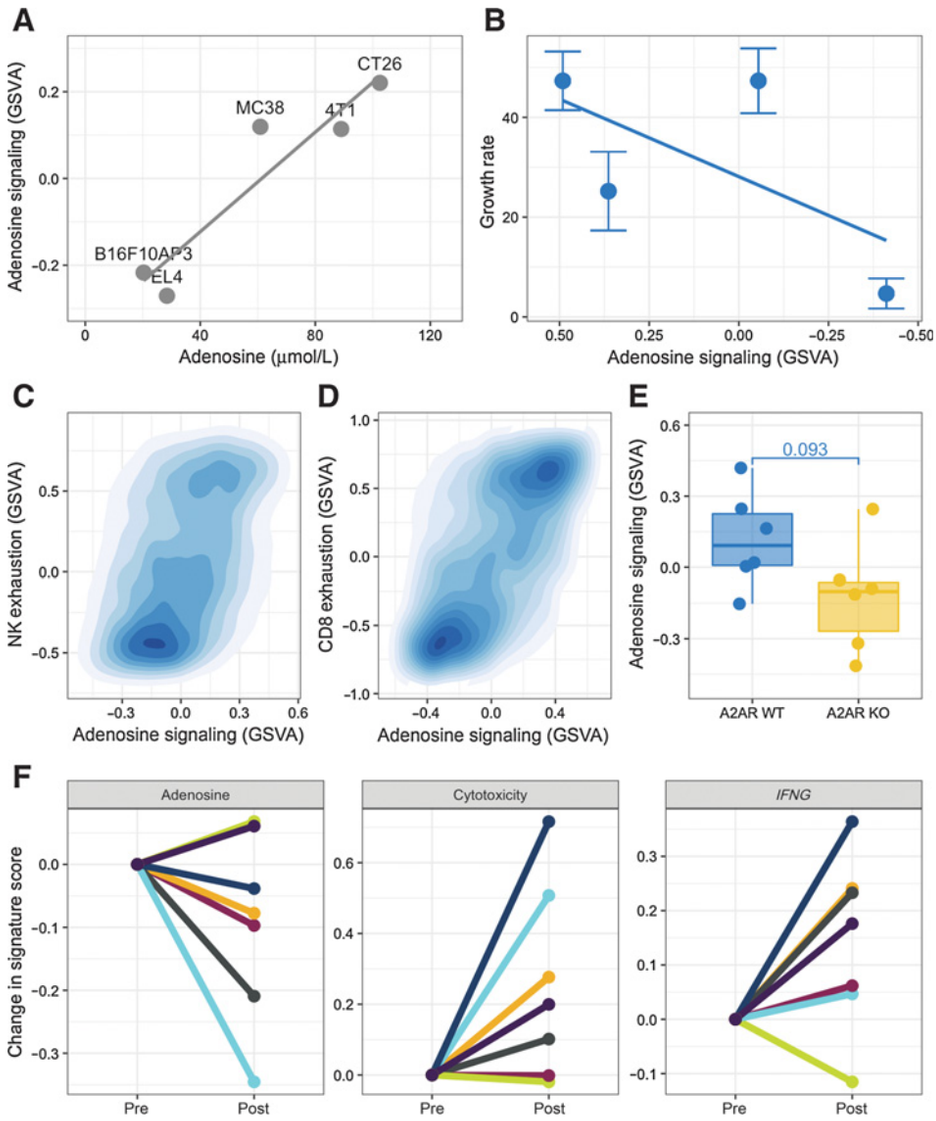

Figure 1.

Signature validation. **A,** The adenosine signaling signature correlates ($r^2 = 0.92$, $P = 0.018$) with absolute adenosine levels in the tumor microenvironment in mouse syngeneic models. **B,** Effective A2AR inhibition, as defined by a reduced growth rate, with a specific small-molecule inhibitor (AZD4635) in the MC38 syngeneic mouse model correlates with reduced adenosine signature scores ($r^2 = -0.62$, $P = 0.001$). **C** and **D,** The adenosine signature correlated with markers of NK-cell exhaustion ($r^2 = 0.4$, $P < 2.2e^{-16}$ and OR = 3.1, $P < 2.2e^{-16}$) and CD8 T-cell exhaustion ($r^2 = 0.6$, $P < 2.2e^{-16}$ and OR = 7.8, $P < 2.2e^{-16}$) in human tumors from TCGA. **E,** Adenosine signaling signature scores are reduced in A2AR KO CD11b$^+$ CD27$^-$ NK cells versus A2AR wild-type NK cells from C57BL/6 mice. **F,** Adenosine signaling scores are reduced in 5 of 7 patients treated with AZD4635 in a phase I trial, 4 of which have concomitant increases in gene expression signatures measuring cytolytic activity and *IFNG* signaling.

using the expression of *NKG7*, *CST7*, *PRF1*, *GZMA*, *GZMB*, and *IFNG*. CD8 exhaustion was determined using the signature provided in ref. (39). *IFNG* signaling was determined using the signature presented by ref. (40).

Genetic associations with adenosine

Genetic associations with adenosine signaling were studied for all genes with a mutation frequency >2% across the cohort being studied and for all copy-number variants. A linear model with tumor type, TMB, and MSI status as covariates was fit to the data, and ANOVA was used to test for significance. Effect sizes were computed as the Cohen's D effect size where the difference between means is normalized for the variance within the data. All *P* values were adjusted for multiple testing using the Benjamini–Hochberg procedure.

Mouse models for signature validation

All animal studies were performed according to AstraZeneca Institutional Animal Care and Use Committee guidelines.

Transcriptional profiling data for the five syngeneic models shown in **Fig. 1A** were obtained from ref. (41). Tumor adenosine measurements from syngeneic models were performed as described in Goodwin and colleagues (42).

For the *in vivo* treatment study shown in **Fig. 1B**, MC38 cells were confirmed free of mycoplasma and mouse pathogens by PCR as part of a rodent pathogen testing panel (IMPACT, IDEXX Bioresearch). Thawed cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% L-glutamine (Sigma Aldrich) at 37°C in a humidified incubator maintained at 5% CO_2. Cell counts were performed prior to implantation by Countess Cell Counter (Invitrogen). For subcutaneous implants, 5×10^{-5} MC38 cells/mouse were resuspended in sterile PBS and injected s.c. into the right flanks of 4- to 6-week-old female C57BL/6 mice (Charles River Labs) in a total volume of 0.1 mL/mouse.

Mice were randomized into treatment groups at a starting tumor volume of 50 to 90 mm^3. AZD4635 nanosuspension formulation (Aptuit, Verona) was reconstituted in sterile water and dosed orally b.i.d. at 50 mg/kg. Tumor volume and body weight were measured

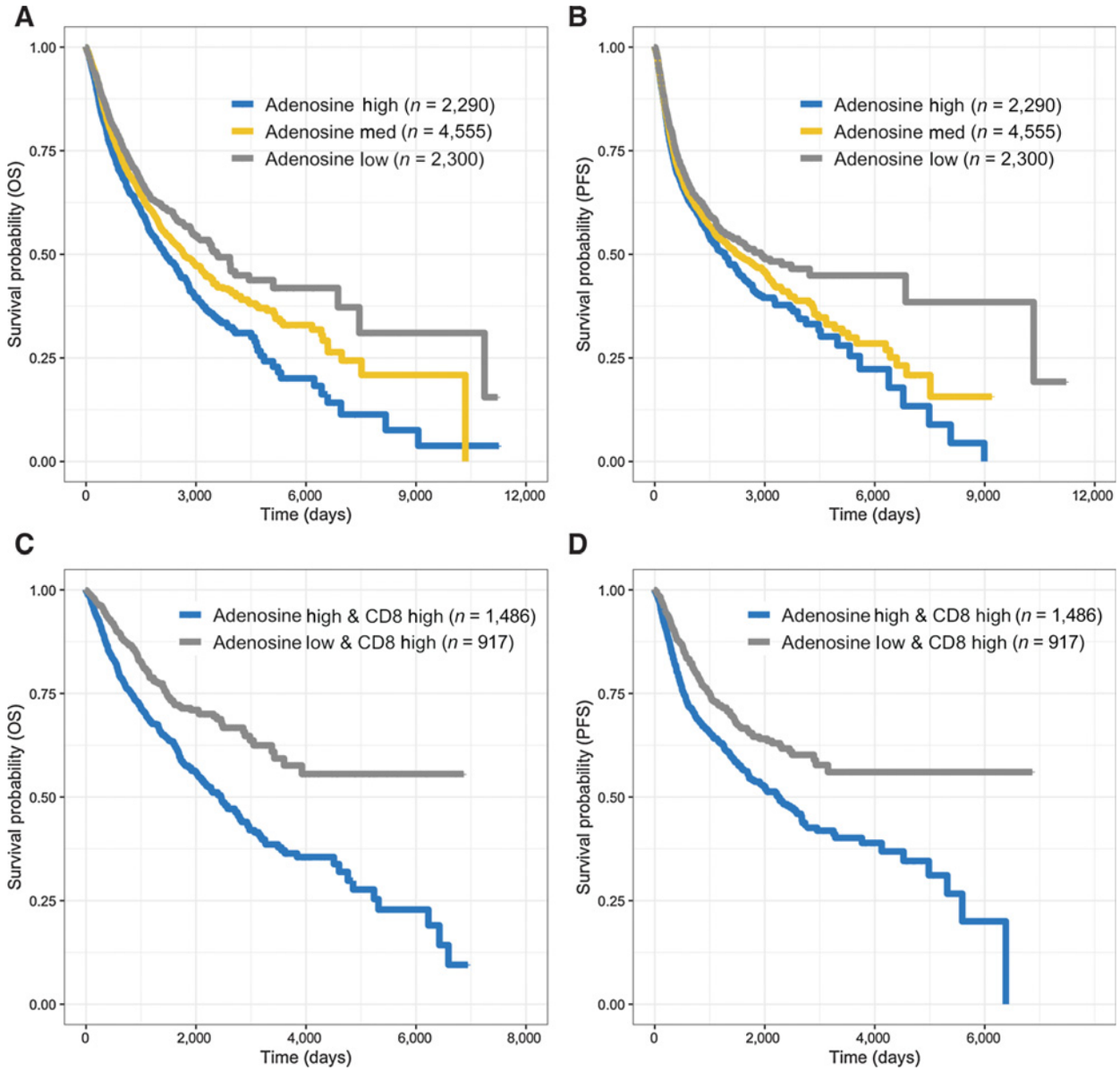

Figure 2.

Adenosine mediates survival in tumors of all types from TCGA. **A,** OS is significantly worse (HR = 0.6, Cox PH $P < 2.2e^{-16}$) in the upper quartile of all tumors from TCGA with the highest levels of adenosine signaling. **B,** The upper quartile also has a significantly worse PFS (HR = 0.77, Cox PH $P = 0.0000006$). **C,** Tumors with a high CD8 infiltrate (greater than the median level of CD8A expression) that are also adenosine high show an OS deficit (HR = 0.47, Cox PH $P < 2.2e^{-16}$) compared with CD8-infiltrated tumors with low adenosine signaling. **D,** Likewise for PFS, tumors that are both CD8 infiltrated and adenosine high have a worse prognosis compared with those that are adenosine low (HR = 0.65, Cox PH $P = 0.0000002$).

twice weekly after randomization. Growth rate was calculated as the slope of a linear model fit to the percent change in tumor volume from day 0 over time.

Human phase IA study of AZD4635

The first-in-human trial, NCT02740985, was conducted to assess safety, pharmacokinetic (PK), and pharmacodynamic activity of AZD4635 as monotherapy and in combination with durvalumab in patients with treatment refractory solid tumors. Predose and on-treatment tumor biopsies within 3 weeks were collected from 7 subjects who were treated with AZD4635 monotherapy. Six subjects (ovarian, sarcoma, pancreatic, colon, myxofibrosarcoma, and cholangiocarcinoma) were treated with 75 mg, whereas the single remaining subject (breast) was treated with 100 mg AZD4635 PO in a once-daily dosing regimen, thus all were at or below the recommended phase 2 dose of 100 mg QD, showed no significant differences in PK, and none of the subjects in this cohort had a RECIST response to AZD4635.

Total RNA was extracted from tumor tissue macrodissected from 5-mm-thick FFPE sections using the miRNeasy FFPE Kit (QIAGEN). RNA integrity and quantity were assessed on the TapeStation 2200

using the RNA ScreenTape System (Agilent). Manufacturer's recommended protocols were followed.

The RNA was subsequently analyzed for gene expression using the NanoString nCounter FLEX Analysis System and the commercially available 770-gene, human PanCancer Immune Profiling Panel (NanoString). Following the manufacturer's standard XT CodeSet Gene Expression Assays protocol, 25 to 100 ng RNA was hybridized with Capture and Reporter probes at 65°C for 22 hours. Posthybridization sample processing on the Prep Station using the high-sensitivity setting was followed by data collection on the Digital Analyzer scanning at 555 fields of view. Preprocessing of the raw count data, which included background subtraction of the negative control probes, positive control normalization, and housekeeping gene normalization, was performed in the nSolver 4.0 (NanoString) software using the geometric means and default parameters. All samples included in downstream analyses fell within the default nSolver QC parameters.

Results

A gene expression signature accurately captures adenosine signaling levels

It is challenging to measure tumor adenosine levels in a high-throughput manner, so we sought to create a gene expression signature that would recapitulate adenosine signaling and allow us to study the wealth of existing data from large collections of tumor transcriptomes. It has previously been shown that causal, or regulatory, protein/gene interaction knowledge is a powerful substrate for the interpretation of transcriptomic data (43–45). We thus sought to compile a regulatory network for the adenosine signaling pathway. Both NLP, as described previously (46–48), and manually extracted knowledge (49) were used to define a network of interactions between the A2AR receptor and downstream entities. Of the four adenosine receptors, A2A was selected as the basis of our study given that A1 and A3 function to increase cAMP rather than decrease it, which is necessary for immune cell suppression (50), whereas A2B has considerably lower affinity for adenosine (50). We focused on regulatory interactions where there was evidence that A2AR increased expression of the downstream entity in the primary scientific literature. We found 172 genes that have been reported to be regulated by A2AR, 90 of which were reported to be positively regulated by A2AR signaling activity. We applied additional filters to ensure the genes are robustly expressed in human tumors and present on a widely used clinical transcriptomics assay. Our final signature consisted of 14 genes whose concordant activity is indicative of adenosine signaling: *PPARG, CYBB, COL3A1, FOXP3, LAG3, APP, CD81, GPI, PTGS2, CASP1, FOS, MAPK1, MAPK3*, and *CREB1*.

To confirm the validity and specificity of our signature, we quantified the intratumoral levels of adenosine in five murine syngeneic models for which we also have transcriptional profiles (41). We find a significant correlation ($r^2 = 0.92$, $P = 0.018$) between measured intratumor adenosine concentrations and adenosine signaling as captured using our signature (**Fig. 1A**). We next assessed whether the adenosine signature tracked with inhibition of the adenosine receptor *in vivo* within the MC38 syngeneic model using AZD4635, an A2AR selective small-molecule currently in clinical development (15, 51). We find that our signature correlates ($r^2 = -0.62$, $P = 0.001$) with reduced growth rate after A2AR inhibition (**Fig. 1B**). Furthermore, knockout of the A2AR receptor (32) abrogated adenosine signaling signature scores in CD11b$^+$ CD27$^-$ NK cells (**Fig. 1E**). A key biological effect of adenosine within human tumors is to suppress immune cell activity (32). In concordance with this, the adenosine signature scores have a significant association with NK-cell ($r^2 = 0.4$, $P < 2.2e^{-16}$ and OR = 3.1, $P < 2.2e^{-16}$, **Fig. 1C**) and CD8$^+$ T-cell ($r^2 = 0.6$, $P < 2.2e^{-16}$ and OR = 7.8, $P < 2.2e^{-16}$, **Fig. 1D**) exhaustion marker expression in TCGA. Finally, 7 patients with a variety of solid tumors were treated once daily with AZD4635 in a phase IA study (NCT02740985) to assess pharmacodynamic changes in signature scores within humans. Adenosine signaling scores were reduced in 5 of the 7 (70%) patients, 4 of which also had concordant increases in gene expression signatures of cytolytic activity and *IFNG* signaling. Taken together, these data demonstrate that our proposed signature is a useful surrogate for adenosine signaling activity when studying bulk transcriptomes of human and mouse tumors.

Adenosine signaling correlates with survival in human disease

Having established that our signature captures adenosine signaling activity within tumors, we next explored the association of adenosine signaling with disease outcomes. Adenosine suppresses a functional antitumor response and so we would expect tumors with a high adenosine drive to be more aggressive and have reduced survival. To confirm this, we used our signature scores to compare survival in tumors with high adenosine signaling with tumors with low adenosine signaling across all cancers in TCGA. However, before doing so, we studied the potential for tumor purity to bias our scores across large datasets. We observed that low purity trended with greater adenosine signature scores (Supplementary Fig. S1A). We therefore established a normalization of signature scores for tumor purity (Supplementary Fig. S1B and Methods) to remove this bias from further studies of human tumors in TCGA.

Adenosine signaling high tumors were defined as the upper quartile of signature scores across all samples, and likewise adenosine low consisted of the lower quartile. We find that high levels of adenosine signaling associate with significantly worse overall survival (OS; HR = 0.6, Cox PH $P < 2.2e^{-16}$) and progression-free survival (PFS; HR = 0.77, Cox PH $P = 0.0000006$) in a pan-cancer model (**Fig. 2A** and **B**). This association remains if the data are split by tertiles (OS HR = 0.75, Cox PH $P = 0.000000006$; PFS HR = 0.83, Cox PH $P = 0.000025$) or on the median (OS HR = 0.81, Cox PH $P = 0.0000002$; PFS HR = 0.86, Cox PH $P = 0.00007$).

Considerable progress has been made in the characterization of the tumor microenvironment from the perspective of immune cell infiltration. However, it remains unclear why some apparently "hot" tumors with an otherwise adequate infiltration of immune cells do not appear to mount an effective antitumor response. We first assessed the relationship of adenosine signaling to immune cell infiltrates inferred from bulk RNA-seq in TCGA using a support vector regression approach based upon the CIBERSORT algorithm (38). There are no strong associations, but we observe weak negative correlations with activated NK-cell and T follicular helper cell scores and a positive correlation with resting NK-cell and macrophage scores (Supplementary Fig. S3A). We therefore studied the ability of adenosine to modulate the activity of existing immune infiltrates by studying only tumors with a high level of CD8$^+$ T-cell infiltration, defined as greater than the median of *CD8A* expression across all samples. We found a dramatic survival deficit in tumors that are both CD8 high and adenosine high versus tumors that are CD8 high but adenosine low, for both OS (HR = 0.47, Cox PH $P < 2.2e^{-16}$) and PFS (HR = 0.65, Cox PH $P = 0.0000002$; **Fig. 2C** and **D**). Further, the survival deficit between adenosine high and low tumors is reduced or ablated in CD8 low tumors (OS Cox PH $P = 0.001$, PFS Cox PH $P = 0.05$).

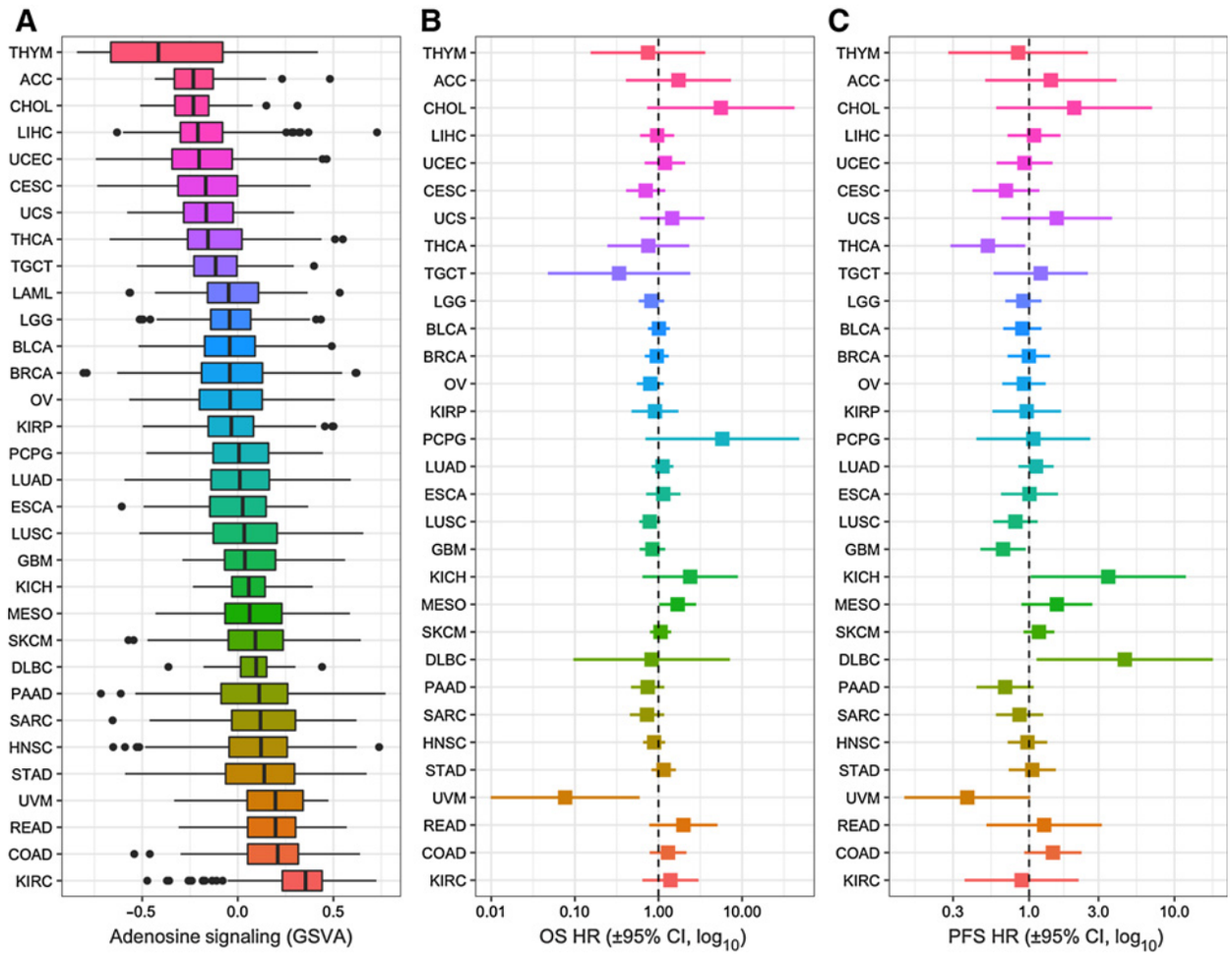

Figure 3.

Adenosine signaling levels vary across tumor types. **A,** Adenosine signaling across the tumor types of TCGA varies and is lowest in thymoma and highest in kidney renal clear cell carcinoma. **B,** Adenosine signaling association with OS in each tumor type from TCGA. **C,** Adenosine signaling association with PFS in each tumor type from TCGA. In **B** and **C**, boxes represent the HR when the upper quartile is compared with the lowest quartile, with whiskers describing the 95% confidence interval (CI).

Adenosine signaling in individual tumor types

We next studied the adenosine signaling profile of each tumor type from TCGA individually. All tumor types exhibit a wide range of adenosine signaling levels, and all have some individuals with high adenosine signaling (**Fig. 3A**). Kidney renal clear cell carcinoma (KIRC) has the highest levels of adenosine signaling on average across all tumor types, whereas thymoma (THYM) has the lowest (**Fig. 3A**). Consistent with this observation, adenosine is known to play an important role within the kidney where it regulates a variety of physiologic functions and is present at significant extracellular concentrations (52). Interestingly, adenosine also plays a role in the thymus, regulating the thymocyte selection process (53).

Concordantly reduced OS and PFS in adenosine high tumors are seen in 13 individual diseases (**Fig. 3C**), with four having an HR < 0.7 for both survival measures: uveal melanoma (UVM, OS HR = 0.08, PFS HR = 0.38), cervical (CESC, OS HR = 0.70, PFS HR = 0.69), pancreatic (PAAD, OS HR = 0.74, PFS HR = 0.68), and thyroid (THCA, OS HR = 0.75, PFS HR = 0.52). However, UVM (HR = 0.08, $P = 0.016$) is the only case where OS is statistically significant for an individual tumor type (**Fig. 3B**). Similarly, glioblastoma (GBM, HR = 0.66, $P = 0.02$), thyroid carcinoma (THCA, HR = 0.52, $P = 0.03$), and UVM (HR = 0.37, $P = 0.05$) are the only diseases where adenosine signaling is statistically associated with worse PFS. Interestingly, diffuse large B-cell lymphoma (DLBCL) tumors with high levels of adenosine signaling are associated with improved PFS (DLBC, HR = 5.19, $P = 0.02$), although notably not concordant with OS.

Genetic correlates of adenosine signaling

Adenosine signaling is not correlated with TMB at a pan-cancer level ($r^2 = 0.02$); however, MSI high tumors have significantly higher levels of adenosine signaling (**Fig. 4C**, $P = 5e^{-16}$). We therefore derived a linear model that incorporated MSI as a covariate with which to identify single-nucleotide variants (SNV) associated with adenosine signaling. Our analysis identifies 23 mutated genes that associate with adenosine signaling (at $q < 0.1$) when all samples are

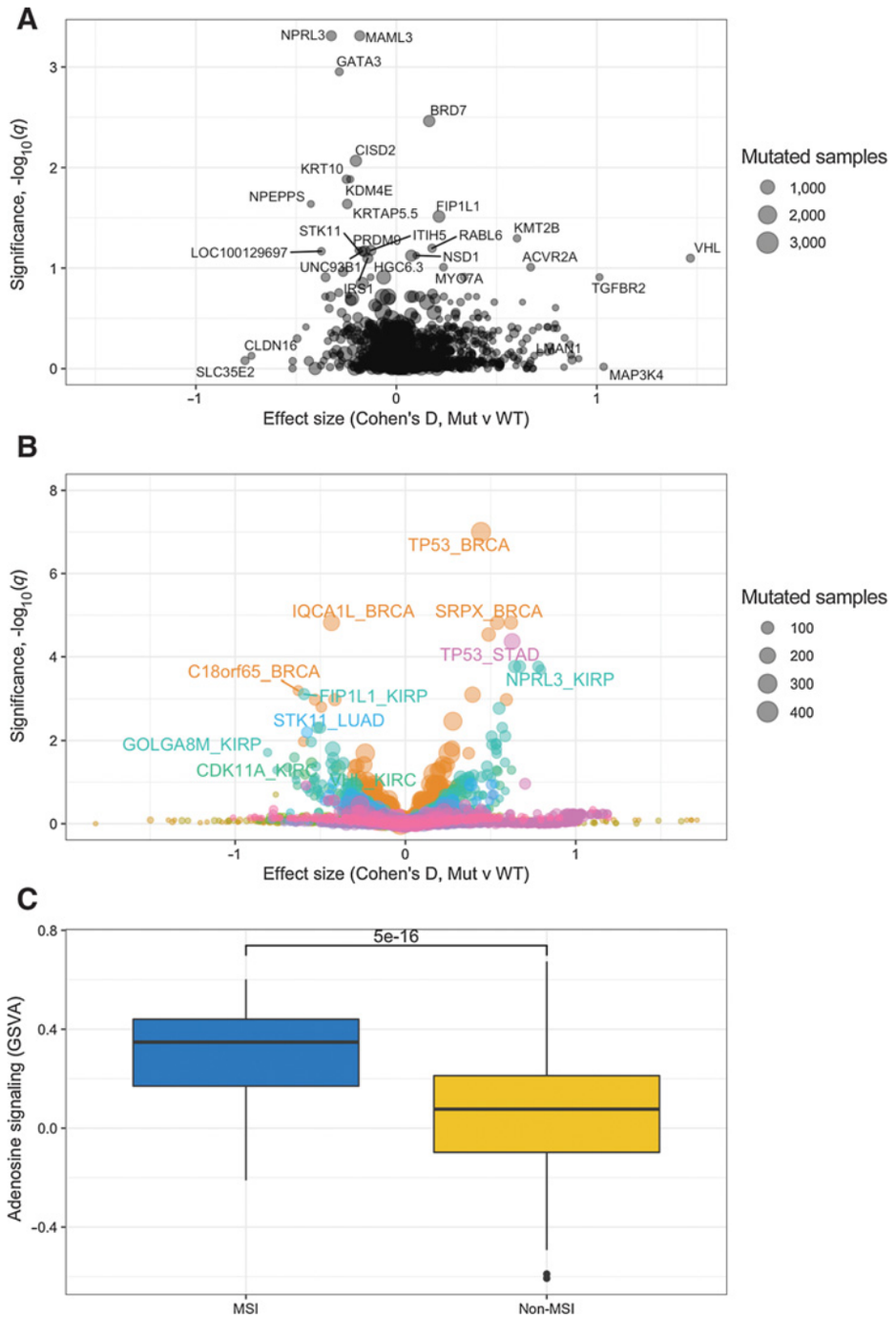

Figure 4.

Genetic correlates of adenosine signaling. **A,** Adenosine signaling in pan-cancer disease segments defined by nonsynonymous mutations at the gene level was compared with non-mutated samples. Multiple testing corrected *P* values (*q*) are shown versus the Cohen's D effect size where values > 0 indicate higher levels in the mutant segment. **B,** As for **A**, but each tumor type was studied independently. **C,** Adenosine signaling in MSI versus non-MSI tumors from TCGA; MSI tumors have significantly higher levels of adenosine signaling.

considered in a pan-cancer model: 9 with enhanced adenosine signaling and 14 with reduced adenosine signaling (**Fig. 4A** and Supplementary Table S1).

Six adenosine-associated genes have an established role in cancer pathogenesis, being members of the cancer gene census (54, 55), including *VHL, ACVR2A, FIP1L1*, and *NSD1* which all correlate with increased adenosine signaling, and *GATA3* and *STK11* that associate with reduced adenosine signaling (Supplementary Fig. S3).

VHL has the largest effect size and is thought to be an E3 ubiquitin ligase that suppresses *HIF1a* expression. Consequently, *VHL* loss-of-function mutations lead to constitutive expression of *HIF1a* which upregulates CD73 and CD39, thereby enhancing the production of adenosine (56). This previously described mechanism gives us further confidence in the relevance of our signature.

GATA3 is an important transcription factor associated with breast cancer and as a key regulator of $CD4^+$ T-cell development with some evidence to suggest its activity is regulated by adenosine in other settings (57).

The tumor suppressor STK11 has recently been shown to drive primary resistance to checkpoint inhibition (58), and the negative

Table 1. Copy-number variants associated with adenosine signaling with Cohen's D effect size > 0.5.

Location	Effect size	*q*	Type	Census genes in locus
chr3 32098168:37495009	1.54	0.0017	DEL	CCR4, MLH1
chr3 1:17201156	1.42	0.0370	DEL	FANCD2, FBLN2, PPARG, RAF1, SRGAP3, VHL, XPC
chr6 119669222:171115067	0.83	0.0413	DEL	ARID1B, BCLAF1, ECT2L, ESR1, EZR, FGFR1OP, LATS1, MLLT4, MYB, PTPRK, QKI, RSPO3, SGK1, TNFAIP3
chr19 39363864:39953130	0.57	0.0096	AMP	none
chr3 12384543:12494277	0.56	0.0000001	AMP	none
chr19 30036025:30321189	0.54	0.0104	AMP	CCNE1
chr19 30183172:30321189	0.54	0.0104	AMP	none
chr1 1:29140747	0.51	0.0012	DEL	ARHGEF10L, ARID1A, CAMTA1, CASP9, ID3, MDS2, MTOR, PAX7, PRDM16, PRDM2, RPL22, SDHB, SKI, SPEN, TNFRSF14
chr1 150637495:150740723	−0.89	0.0104	AMP	none
chr1 228801039:249250621	−0.90	0.0099	AMP	AKT3, FH, RGS7
chr8 113630879:139984811	−0.94	0.0017	AMP	CSMD3, EXT1, FAM135B, MYC, NDRG1, RAD21

association with adenosine signaling identified here most likely reflects the immunologically cold/excluded tumor microenvironment for which an immunosuppressive phenotype has not been activated. This raises the interesting possibility that the other negatively associated genetic segments might also exhibit resistance to immunotherapy. Notably, the most significantly associated genetic mutations are in *NPRL3* which is part of the GATOR1 complex, which, like LKB1 via AMPK, feeds into the mTOR signaling pathway (59, 60).

We found 55 SNVs associated with adenosine within an individual tumor type ($q < 0.05$, **Fig. 4B** and Supplementary Table S2), comprising 25 from kidney renal papillary cell carcinoma, 23 from breast cancer, 3 from kidney renal clear cell carcinoma, and 1 each from lung adenocarcinoma (*STK11*), prostate adenocarcinoma (*RABL6*), stomach adenocarcinoma (*TP53*), and head and neck squamous cell carcinoma (*BRD7*). Seven of these associations feature cancer census genes: *TP53* in BRCA and STAD, *GATA3* in BRCA, *CDH1* in BRCA, *VHL* in KIRC, *FIP1L1* in KIRP, and *STK11* in LUAD (Supplementary Fig. S4).

Somatic copy-number alterations (SCNA) are also associated with adenosine signaling. Note that 124 SCNA are significant ($q < 0.05$) with 11 having an effect size greater than 0.5 (**Table 1** and Supplementary Fig. S5). This includes a deletion on chromosome 3 which removes *VHL* and replicates the observation seen with SNVs.

Adenosine signaling is associated with TGFβ

TGFBR2 and *ACVR2A* mutations are among the most significant associations with adenosine levels in a pan-cancer model even after correction for MSI status. Both are members of the TGFβ superfamily encoding the TGFβ receptor and the structurally related activin growth factor receptor, respectively. TGFβ signaling has a complex and highly context-dependent association with cancer biology. As a tumor suppressor, TGFβ mutation promotes tumorigenesis, but its loss has also been shown to increase chemokine signaling resulting in infiltration of myeloid-derived suppressor cells which themselves produce TGFβ and eventually drive immunosuppression, thereby promoting tumor growth (61). Our result raises the possibility that this suppression is driven largely through the adenosine axis.

To further explore this relationship, we conducted a deeper study of the association between adenosine and TGFβ. Thorsson and colleagues (62) defined six primary immune subtypes of cancer including a TGFβ-dominant group, cluster 6 ("C6"). We find that adenosine signaling is significantly higher in this group compared with the other five immune subtypes (**Fig. 5A**). We further expanded our analysis to include the 43 members of the TGFβ superfamily (63) and find that mutations in any of these genes are associated with a higher level of adenosine signaling (**Fig. 5B**). Finally, tumors that are both adenosine high and mutant in a TGFβ superfamily member have worse OS compared with tumors that are adenosine low and TGFβ wild-type (HR = 0.43, $P < 2.2e^{-16}$), or those that are either TGFβ mutant (HR = 0.74) or adenosine high (HR = 0.72; **Fig. 5C**).

Adenosine signaling is prognostic for immunotherapy response

To test the clinical utility of the signature and the extent to which adenosine affects immune checkpoint therapy, we studied cohorts of patients treated with checkpoint inhibitors. Prat and colleagues generated gene expression profiles of 65 patients from a variety of solid tumors that were treated with anti-PD1 therapy (35). Chen and colleagues profiled 53 patients with metastatic melanoma that were treated with anti–CTLA-4 therapy (36). We find that responders to immune checkpoint therapy, as classified by their best overall response, have lower levels of baseline tumor adenosine signaling than do patients which progress on both anti-PD1 therapy (**Fig. 6A**) and anti–CTLA-4 therapy (**Fig. 6C**). We used logistic regression to model the probability of a patient being a complete responder (CR), partial responder (PR), or having stable disease (SD) versus progressive disease (PD) in these cohorts. A signature score just below 0 (−0.01368) equates to a 50% probability of being a responder, and a signature score of −0.4 equates to a 75% probability of being a responder (Supplementary Fig. S7).

In the anti–CTLA-4 dataset, only 6 genes from our 14-gene signature are present on the panel used. To study the effect this might have we scored the anti-PD1 dataset with the same 6 genes. The overall trend of results is retained, but the sensitivity of the signature is reduced (PD vs. SD 6 gene $P = 0.072$ vs. 14 gene $P = 0.076$, and PD vs. PR/CR 6 gene $P = 0.13$ vs. 14 gene $P = 0.0027$).

There is also a highly significant association between adenosine signaling at baseline and PFS on anti-PD1 therapy (**Fig. 6B**; HR = 0.29, Cox PH $P = 0.00012$). Interestingly, expression of the gene encoding PDL1 (CD274), which is highly correlated with PDL1 IHC measurements (35), does not associate with PFS in the same dataset (HR = 0.8, Cox PH $P = 0.47$). Furthermore, combining adenosine and CD274 expression does not enhance the ability to predict immunotherapy response beyond adenosine alone (**Fig. 6B** and Supplementary Fig. S6). These results would suggest that baseline levels of adenosine

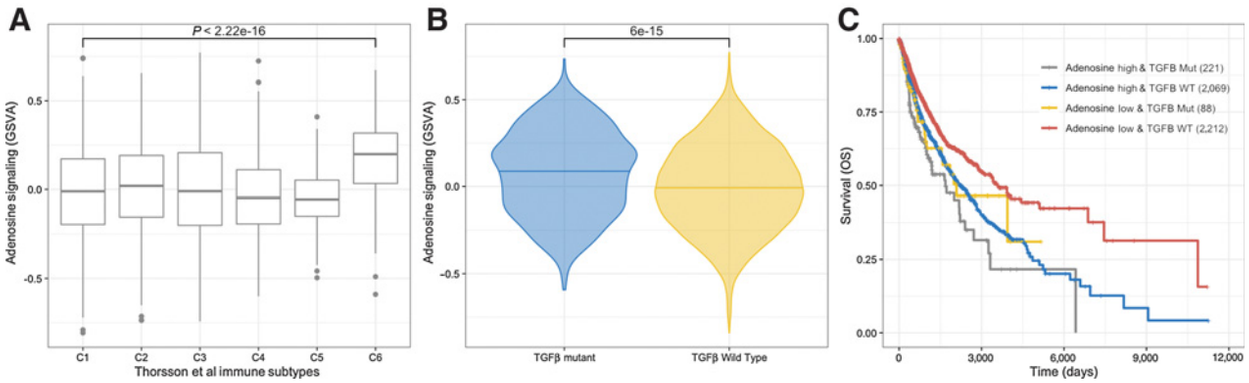

Figure 5.

Adenosine signaling associates with TGFβ. **A,** Adenosine signaling levels are significantly higher in the TGFβ-driven tumor cluster (C6) from Thorsson and colleagues. **B,** Tumors from TCGA mutated in one of the 43 TGFβ superfamily members have higher levels of adenosine signaling versus TGFβ superfamily wild-type tumors. **C,** Tumors that are adenosine high and TGFβ superfamily mutant have worse OS compared with tumors that are adenosine low and TGF-β wild-type (HR = 0.43, Cox PH $P < 2.2e^{-16}$), or those that are either TGFβ mutant (HR = 0.74) or adenosine high (HR = 0.72).

are an important correlate of response to immunotherapy and that our signature might complement PDL1 as a marker in this regard for the existing checkpoint inhibitors and potentially as A2AR inhibitors progress through the clinic.

Discussion

We sought to characterize the pan-cancer role of adenosine in human tumors, to test the hypothesis that adenosine signaling has a negative prognostic link within human tumors, and to identify segments of disease where this might be most pronounced. We therefore report the derivation and validation of a gene expression signature that recapitulates adenosine signaling and with which we address these questions.

Our data show that adenosine signaling levels vary across the tumor types of TCGA and that this is associated with suppression of anti-tumor immunity in tumors where an otherwise adequate $CD8^+$ T-cell infiltrate is present. This observation has important implications for the way in which we model and identify functional antitumor immune responses. Significant progress has been made in the identification of

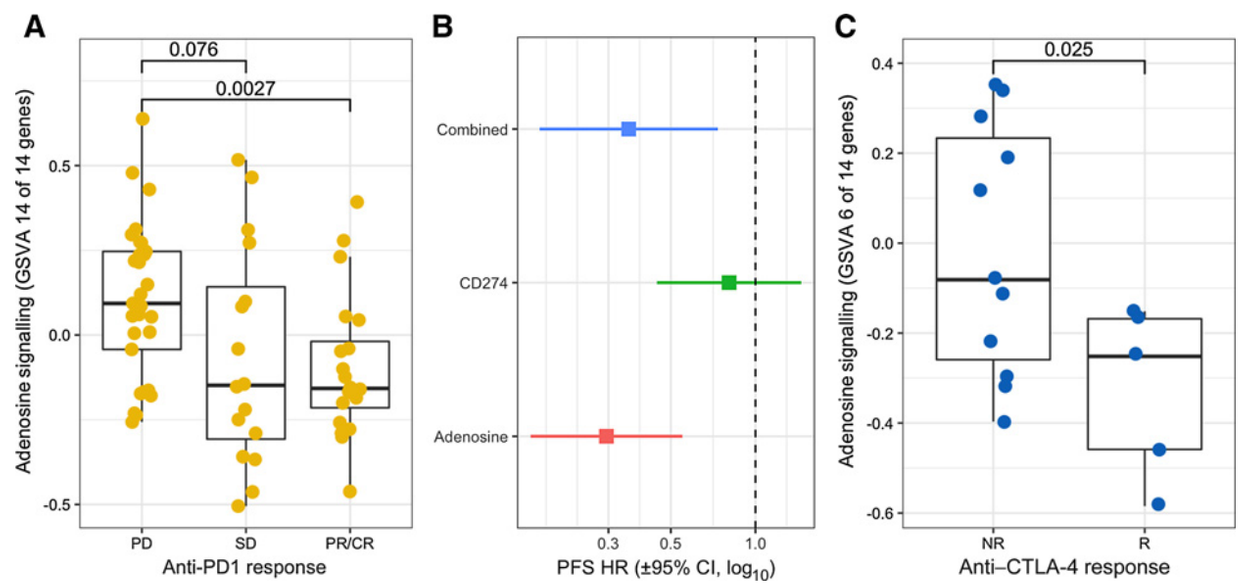

Figure 6.

Adenosine signaling is predictive for response to immunotherapy. **A,** Baseline tumor expression profiles from patients with a variety of solid tumors are higher in progressors versus responders to anti-PD1 therapy (either pembrolizumab or nivolumab) from Prat and colleagues (35). **B,** On-treatment PFS is also significantly reduced in adenosine signaling high tumors (HR = 0.29, Cox PH $P = 0.00012$) but not in CD274 mRNA high tumors (HR = 0.8, Cox PH $P = 0.47$). Combining adenosine signature score and CD274 expression does not improve prognosis compared with the adenosine signature alone. **C,** Baseline tumor expression profiles from patients with metastatic melanoma are higher in nonresponders from Chen and colleagues (36) despite only 6 genes from our 14 gene signature being present on the panel used.

immune infiltrates alone that associate with outcomes (e.g., the Immunoscore; ref. 64), yet our results suggest that orthogonal measures of immunosuppressive effectors could enhance these measures.

We also identify genetic segments of disease that associate with higher adenosine signaling including MSI tumors and specific genetic variation in TGFβ. These mutations have potential as markers for adenosine-targeted therapies and are consistent with the concept that adenosine signaling acts to suppress the inflammatory response to highly immunogenic tumors (65). The relationship between adenosine signaling and TGFβ associates adenosine with fibroblast biology and reflects early clinical data from the anti-CD73 monoclonal antibody Oleclumab (ASCO) in pancreatic cancer, an indication known to be rich in cancer-associated fibroblasts (66).

Finally, we demonstrate that adenosine correlates with survival across tumors of all types and within specific indications. Furthermore, baseline adenosine signaling scores appear to predict response to immune checkpoint therapies. We show that this is independent of PDL1 expression and that adenosine signaling does not correlate with TMB. Further investigation is therefore warranted to establish the potential utility of adenosine signaling as an additional measure to identify patients likely to respond to checkpoint therapy. In addition, these findings support the development of adenosine targeting agents for use in combination with existing checkpoint inhibitors. However, it requires further clinical validation using an agent targeting the adenosine axis to be considered predictive of response to this class of agents as opposed to prognostic in the presence of intact adenosine signaling. As our signature has been derived independently of any specific molecular agent targeting the adenosine pathway, it may have utility across a broad spectrum of candidate drugs currently in development.

Our results present several unexpected findings. First, the CT26 mouse model and MSI high human tumors are sensitive to immune checkpoint inhibitors, while the MC38 mouse model has shown variable responsiveness to immune checkpoint inhibition, yet we find both associated with high adenosine signaling (**Figs. 1A** and **4C**). In addition, not all tumor types with high adenosine signaling on average appear to exhibit a survival deficit. Further, although reduced adenosine signaling enriches for responders to checkpoint inhibition, not all adenosine low patients respond and vice versa. Immune checkpoint inhibitor sensitivity is likely determined by many factors in addition to adenosine. For example, the presence of $CD8^+$ T cells, expression of PDL1, and high TMB are all associated with checkpoint response (67, 68). It is also likely that an immune infiltration/response must occur prior to a state of adenosine-mediated repression. As such, adenosine is another factor that contributes to the balance between those that induce antitumor immunity and those that are immunosuppressive. Further works to characterize the tipping point in this balance are necessary.

The signature described here may be limited by the decision to only include genes within a commercially available RNA expression panel. This decision ensures the translatability of the signature to ongoing clinical studies as well as direct comparison with other reported gene expression systems (69, 70). However, expansion and further development of the signature using a broader panel of transcripts could enhance the sensitivity of the signature. A second area which falls outside of the scope of this report but may well be relevant here involves other branches of adenosine biology beyond A2AR. For example, inflammatory signaling through ATP (71) or other nodes of the larger adenine nucleotide signaling axis (72). Finally, a group from Corvus Pharmaceuticals has taken an orthogonal approach to generating an adenosine-related gene signature. Here, the authors identified genes upregulated by NECA, an adenosine analog, and suppressed by CPI-444, an A2AR antagonist (69). The two signatures have just one gene in common (*PTGS2*) which may reflect the compound-specific nature of the CPI-444 signature.

In conclusion, the expression signature reported here is a useful clinical tool that establishes the adenosine pathway as a key mediator of a successful antitumor immune response, demonstrates the prognostic potential for existing immunotherapies of quantifying adenosine drive, and informs patient selection strategies for adenosine pathway modulators currently in development.

Disclosure of Potential Conflicts of Interest

B. Sidders, P. Zhang, D.L. Russell, A. Borodovsky, J. Armenia, J.R. Dry, and K. Sachsenmeier are employees/paid consultants for AstraZeneca. T.M. Bauer is an employee/paid consultant for Pfizer, Blueprint Medicines, Exelixis, Bayer, LOXO Oncology, and Guardant Health, and reports receiving speakers bureau honoraria from L&M Healthcare. G.S. Falchook is an employee/paid consultant for Fujifilm and EMD Serono, reports receiving commercial research grants from 3-V Biosciences, Abbisko, Abbvie, ADC therapeutics, Aileron, American Society of Clinical Oncology, Amgen, ARMO, AstraZeneca, BeiGene, Bioatla, Biothera, Celldex, Celgene, Ciclomed, Curegenix, Curis, Cyteir, Daiichi, DelMar, eFFECTOR, Eli Lilly, EMD Serono, Epizyme, Exelixis, Fujifilm, Genmab, GlaxoSmithKline, Hutchison MediPharma, Igntya, Jacobio, Jounce, Kolltan, Loxo, Medimmune, Millennium, Merck, miRNA therapeutics, National Institutes of Health, Novartis, OncoMed, Oncothyreon, Precision Oncology, Prelude, Regeneron, Rgenix, Ribon, Strategia, Syndax, Taiho, Takeda, Tesaro, Tocagen, Turning point therapeutics, U.T. MD Anderson Cancer Center, Vegenics, and Xencor, speakers bureau honoraria from Total Health Conferencing, and other remuneration from Wolters Kluwer, Bristol-Myers Squibb, EMD Serono, Fujifilm, Milennium, and Sarah Cannon Research Institute. M. Merchant is an employee/paid consultant for AstraZeneca and Epizyme. G. Pouliot is an employee/paid consultant for, reports receiving commercial research grants from, and holds ownership interest (including patents) in AstraZeneca. J.C. Barrett and R. Woessner are employees/paid consultants for and hold ownership interest (including patents) in AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: B. Sidders, M. Merchant, J.C. Barrett, J.R. Dry, R. Woessner, K. Sachsenmeier

Development of methodology: B. Sidders, K. Goodwin, M. Merchant, J.C. Barrett, J.R. Dry, K. Sachsenmeier

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Goodwin, G. O'Connor, A. Borodovsky, J. Armenia, J.C. Bendell, T.M. Bauer, M.R. Patel, G.S. Falchook, M. Merchant, J.R. Dry

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Sidders, P. Zhang, J. Armenia, R. McEwen, B. Linghu, M.R. Patel, M. Merchant, G. Pouliot, J.C. Barrett, J.R. Dry, R. Woessner, K. Sachsenmeier

Writing, review, and/or revision of the manuscript: B. Sidders, P. Zhang, D.L. Russell, J. Armenia, B. Linghu, J.C. Bendell, T.M. Bauer, M.R. Patel, M. Merchant, G. Pouliot, J.R. Dry, R. Woessner, K. Sachsenmeier

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.L. Russell, R. McEwen, R. Woessner, K. Sachsenmeier

Study supervision: M.R. Patel, M. Merchant, G. Pouliot, J.R. Dry, R. Woessner

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References

1. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
2. Serrano P, Hartmann M, Schmitt E, Franco P, Amexis G, Gross J, et al. Clinical development and initial approval of novel immune checkpoint inhibitors in oncology: insights from a global regulatory perspective. Clin Pharmacol Ther 2019;105:582–97.
3. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018;359:1361–5.
4. Boyle GM. Therapy for metastatic melanoma: an overview and update. Expert Rev Anticancer Ther 2011;11:725–37.
5. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med 2018;378:2078–92.
6. Landoni E, Savoldo B. Treating hematological malignancies with cell therapy: where are we now? Expert Opin Biol Ther 2018;18:65–75.
7. Wargo JA, Cooper ZA, Flaherty KT. Universes collide: combining immunotherapy with targeted therapy for cancer. Cancer Discov 2014;4:1377–86.
8. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science 2015;124–8.
9. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N Engl J Med 2016;375:1823–33.
10. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell 2018;33:843–52.e4.
11. Vijayan D, Young A, Teng MWL, Smyth MJ. Targeting immunosuppressive adenosine in cancer. Nat Rev Cancer 2017;17:709–24.
12. Young A, Mittal D, Stagg J, Smyth MJ. Targeting cancer-derived adenosine: new therapeutic approaches. Cancer Discov 2014;4:879–88.
13. Ohta A, Gorelik E, Prasad SJ, Ronchese F, Lukashev D, Wong MKK, et al. A2A adenosine receptor protects tumors from antitumor T cells. Proc Natl Acad Sci U S A 2006;103:13132.
14. Willingham SB, Ho PY, Hotson A, Hill C, Piccione EC, Hsieh J, et al. A2AR antagonism with CPI-444 induces antitumor responses and augments efficacy to anti-PD-(L)1 and anti-CTLA-4 in preclinical models. Cancer Immunol Res 2018;6:1136–49.
15. Borodovsky A, Wang Y, Ye M, Shaw JC, Sachsenmeier KF, Deng N, et al. Preclinical pharmacodynamics and antitumor activity of AZD4635, a novel adenosine 2A receptor inhibitor that reverses adenosine mediated T cell suppression [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2017; 2017 Apr 1–5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl):Abstract nr 5580.
16. Pinna A. Adenosine A2A receptor antagonists in Parkinson's disease: progress in clinical trials from the newly approved istradefylline to drugs in early development and those already discontinued. CNS Drugs 2014;28:455–74.
17. Houthuys E, Marillier R, Deregnaucourt T, Brouwer M, Basilico P, Pirson R, et al. EOS100850, an insurmountable and non-brain penetrant A2A receptor antagonist, inhibits adenosine-mediated T cell suppression, demonstrates anti-tumor activity and exhibits best-in class characteristics [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl): Abstract nr LB-291.
18. Galezowski M, Wegrzyn P, Bobowska A, Commandeur C, Dziedzic K, Nowogrodzki M, et al. Characterization of novel dual A2A/A2B adenosine receptor antagonists for cancer immunotherapy [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl):Abstract nr 3770.
19. Emens L, Powderly J, Fong L, Brody J, Forde P, Hellmann M, et al. CPI-444, an oral adenosine A2a receptor (A2aR) antagonist, demonstrates clinical activity in patients with advanced solid tumors [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2017; 2017 Apr 1–5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl):Abstract nr CT119.
20. Chiappori A, Williams CC, Creelan BC, Tanvetyanon T, Gray JE, Haura EB, et al. Phase I/II study of the A2AR antagonist NIR178 (PBF-509), an oral immunotherapy, in patients (pts) with advanced NSCLC. J Clin Oncol 2018;9089.
21. De Henau, Olivier. First-in-human study of EO100850 in patients with cancer [Internet]. U.S. National Library of Medicine: 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT03873883.
22. Beavis PA, Henderson MA, Giuffrida L, Mills JK, Sek K, Cross RS, et al. Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. J Clin Invest 2017;127:929–41.
23. Arab S, Kheshtchin N, Ajami M, Ashurpoor M, Safvati A, Namdar A, et al. Increased efficacy of a dendritic cell-based therapeutic cancer vaccine with adenosine receptor antagonist and CD73 inhibitor. Tumour Biol 2017;39: 1010428317695021.
24. Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. J Immunother Cancer. 2018;6:57.
25. Fong L, Hotson A, Powderly J, Sznol M, Heist RS, Choueiri TK, et al. Adenosine A2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. Cancer Discov 2019;10:40–53.
26. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 2013;14:7.
27. Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann O, McEwen R, et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic Acids Res 2016;44:e108.
28. Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. Cell 2018;173:291–304.e6.
29. Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, et al. An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. Cell 2018;173:400–16.e11.
30. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic signaling pathways in the cancer genome atlas. Cell 2018;173:321–37.e10.
31. Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, et al. Genomic and functional approaches to understanding cancer aneuploidy. Cancer Cell 2018;33: 676–89.e3.
32. Young A, Ngiow SF, Gao Y, Patch A-M, Barkauskas DS, Messaoudene M, et al. A2AR adenosine signaling suppresses natural killer cell maturation in the tumor microenvironment. Cancer Res 2018;78:1003–16.
33. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods 2015;12:357–60.
34. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 2017;14:417–9.
35. Prat A, Navarro A, Paré L, Reguart N, Galván P, Pascual T, et al. Immune-related gene expression profiling after PD-1 blockade in non-small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Cancer Res 2017;77: 3540–50.
36. Chen P-L, Roh W, Reuben A, Cooper ZA, Spencer CN, Prieto PA, et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. Cancer Discov 2016;6:827–37.
37. Therneau TM. Survival analysis [R package survival version 2.44-1.1]. Comprehensive R Archive Network (CRAN) [cited 2019 May 15]. Available from: https://CRAN.R-project.org/package=survival.
38. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015; 12:453–7.
39. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, et al. Gene expression markers of tumor infiltrating leukocytes. J Immunother Cancer. 2017; 5:18.
40. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest 2017;127:2930–40.
41. Mosely SIS, Prime JE, Sainson RCA, Koopmann J-O, Wang DYQ, Greenawalt DM, et al. Rational selection of syngeneic preclinical tumor models for immunotherapeutic drug discovery. Cancer Immunol Res 2016;5:29–41.
42. Goodwin KJ, Gangl E, Sarkar U, Pop-Damkov P, Jones N, Borodovsky A, et al. Development of a quantification method for adenosine in tumors by LC-MS/MS with dansyl chloride derivatization. Anal Biochem 2019;568:78–88.
43. Chindelevitch L, Ziemek D, Enayetallah A, Randhawa R, Sidders B, Brockel C, et al. Causal reasoning on biological networks: interpreting transcriptional changes. Bioinformatics 2012;28:1114–21.
44. Fakhry CT, Choudhary P, Gutteridge A, Sidders B, Chen P, Ziemek D, et al. Interpreting transcriptional changes using causal graphs: new

methods and their practical utility on public networks. BMC Bioinformatics 2016;17:318.
45. Sidders B, Brockel C, Gutteridge A, Harland L, Jansen PG, McEwen R, et al. Precompetitive activity to address the biological data needs of drug discovery. Nat Rev Drug Discov 2014;13:83–4.
46. Jamieson DG, Roberts PM, Robertson DL, Sidders B, Nenadic G. Cataloging the biomedical world of pain through semi-automated curation of molecular interactions. Database 2013;2013:bat033.
47. Jamieson DG, Moss A, Kennedy M, Jones S, Nenadic G, Robertson DL, et al. The pain interactome: connecting pain-specific protein interactions. Pain 2014;155:2243–52.
48. Biorelate - curating truths in biomedicine [Internet]. [cited 2020 Feb 4]. Available from: https://www.biorelate.com/.
49. Ingenuity pathway analysis - QIAGEN bioinformatics [Internet]. QIAGEN bioinformatics. [cited 2020 Feb 4]. Available from: https://www.qiagenbioinformatics.com/?qia-storyline=products/ingenuity-pathway-analysis.
50. Sheth S, Brito R, Mukherjea D, Rybak L, Ramkumar V. Adenosine receptors: expression, function and regulation [Internet]. Int J Mol Sci 2014;15:2024–52. Available from: http://dx.doi.org/10.3390/ijms15022024.
51. Borodovsky A, Wang Y, Ye M, Shaw JC, Sachsenmeier K, Deng N, et al. Inhibition of A2AR by AZD4635 induces anti-tumor immunity alone and in combination with anti-PD-L1 in preclinical models [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2018;78(13 Suppl): Abstract nr 3751.
52. Vallon V, Mühlbauer B, Osswald H. Adenosine and kidney function. Physiol Rev 2006;86:901–40.
53. Köröskényi K, Joós G, Szondy Z. Adenosine in the thymus. Front Pharmacol 2017;8:932.
54. Futreal PA, Andrew Futreal P, Coin L, Marshall M, Down T, Hubbard T, et al. A census of human cancer genes. Nat Rev Cancer 2004;4:177–83.
55. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45:D777–83.
56. Hatfield SM, Kjaergaard J, Lukashev D, Belikoff B, Schreiber TH, Sethumadhavan S, et al. Systemic oxygenation weakens the hypoxia and hypoxia inducible factor 1α-dependent and extracellular adenosine-mediated tumor protection. J Mol Med 2014;92:1283–92.
57. Ahmad SF, Ansari MA, Nadeem A, Bakheet SA, Almutairi MM, Attia SM. Adenosine A2A receptor signaling affects IL-21/IL-22 cytokines and GATA3/T-bet transcription factor expression in CD4 T cells from a BTBR T Itpr3tf/J mouse model of autism. J Neuroimmunol 2017;311:59–67.
58. Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarincoma. Cancer Discov 2018;8:822–35.
59. Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell 2004;6:91–9.
60. Shen K, Huang RK, Brignole EJ, Condon KJ, Valenstein ML, Chantranupong L, et al. Architecture of the human GATOR1 and GATOR1-Rag GTPases complexes. Nature 2018;556:64–9.
61. Yang L, Pang Y, Moses HL. TGF-β and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol 2010;31:220–7.
62. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang T-H, et al. The immune landscape of cancer. Immunity 2018;48:812–30.e14.
63. Korkut A, Zaidi S, Kanchi RS, Rao S, Gough NR, Schultz A, et al. A pan-cancer analysis reveals high-frequency genetic alterations in mediators of signaling by the TGF-β superfamily. Cell Syst 2018;7:422–37.e7.
64. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018;391:2128–39.
65. Kjaergaard J, Hatfield S, Jones G, Ohta A, Sitkovsky M. A adenosine receptor gene deletion or synthetic a antagonist liberate tumor-reactive CD8 T cells from tumor-induced immunosuppression. J Immunol 2018;201:782–91.
66. von Ahrens D, Bhagat TD, Nagrath D, Maitra A, Verma A. The role of stromal cancer-associated fibroblasts in pancreatic cancer. J Hematol Oncol 2017;10:76.
67. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol 2016;17:e542–51.
68. Samstein RM, Lee C-H, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 2019;51:202–6.
69. Willingham S, Hotson AN, Laport G, Kwei L, Fong L, Sznol M, Powderly J, Miller R. 1137PD: Identification of adenosine pathway genes associated with response to therapy with the adenosine receptor antagonist CPI-444. Ann Oncol 2018;29:8.
70. Kim E, Palackdharry S, Yaniv B, Mark J, Tang A, Wilson K, et al. Gene expression signature after one dose of neoadjuvant pembrolizumab associated with tumor response in head and neck squamous cell carcinoma (HNSCC). J Clin Oncol 2018;6059.
71. Draganov D, Gopalakrishna-Pillai S, Chen Y-R, Zuckerman N, Moeller S, Wang C, et al. Modulation of P2X4/P2X7/pannexin-1 sensitivity to extracellular ATP via Ivermectin induces a non-apoptotic and inflammatory form of cancer cell death. Sci Rep 2015;5:16222.
72. Fliegert R, Heeren J, Koch-Nolte F, Nikolaev VO, Lohr C, Meier C, et al. Adenine nucleotides as paracrine mediators and intracellular second messengers in immunity and inflammation. Biochem Soc Trans 2019;47:329–37.