



Long-term Benefit of PD-L1 Blockade in Lung Cancer Associated with JAK3 Activation

Citation

Van Allen, E. M., H. G. Golay, Y. Liu, S. Koyama, K. Wong, A. Taylor-Weiner, M. Giannakis, et al. 2015. "Long-Term Benefit of PD-L1 Blockade in Lung Cancer Associated with JAK3 Activation." *Cancer Immunology Research* 3 (8) (May 26): 855–863.
doi:10.1158/2326-6066.cir-15-0024.

Published version

<https://doi.org/10.1158/2326-6066.CIR-15-0024>

Link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:32706163>

Terms of use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Open Access Policy Articles (OAP), as set forth at

<https://harvardwiki.atlassian.net/wiki/external/NGY5NDE4ZjgzNTc5NDQzMGIzZWZhMGFIOWI2M2EwYTg>

Accessibility

<https://accessibility.huit.harvard.edu/digital-accessibility-policy>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#)



Published in final edited form as:

Cancer Immunol Res. 2015 August ; 3(8): 855–863. doi:10.1158/2326-6066.CIR-15-0024.

Long-term benefit of PD-L1 blockade in lung cancer associated with JAK3 activation

Eliezer M. Van Allen^{1,2,*}, Hadrien G. Golay^{1,*}, Yan Liu^{1,*}, Shohei Koyama¹, Karrie Wong¹, Amaro Taylor-Weiner², Marios Giannakis^{1,2}, Maegan Harden², Vanesa Rojas-Rudilla⁴, Aaron Chevalier², Tran Thai¹, Christine Lydon¹, Stacy Mach¹, Joshua A. Wong¹, Alexandra R. Rabin¹, Joshua Helmkamp¹, Lynette Sholl⁴, Scott L. Carter², Geoffrey Oxnard¹, Pasi Janne^{1,3}, Gad Getz^{2,5}, Neal Lindeman⁴, Peter S. Hammerman^{1,2}, Levi A. Garraway^{1,2}, F. Stephen Hodi^{1,6}, Scott Rodig^{4,6}, Glenn Dranoff^{1,6}, Kwok-Kin Wong^{1,3,**}, and David A. Barbie^{1,3,**}

¹Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, Massachusetts 02215, USA

²Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, Massachusetts 02142, USA

³Belfer Institute for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, MA

⁴Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

⁵Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

⁶Center for Immuno-Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Abstract

PD-1 immune checkpoint blockade occasionally results in durable clinical responses in advanced metastatic cancers. However, mechanism-based predictors of response to this immunotherapy remain incompletely characterized. We performed comprehensive genomic profiling on a tumor and germline sample from a patient with refractory lung adenocarcinoma who achieved marked long-term clinical benefit from anti-PD-L1 therapy. We discovered activating somatic and germline amino acid variants in *JAK3* that promoted PD-L1 induction in lung cancer cells and in the tumor immune microenvironment. These findings suggest that genomic alterations that

Corresponding Authors: David Barbie, MD PhD, Dana-Farber Cancer Institute, 450 Brookline Avenue D819, Boston, MA 02215, dbarbie@partners.org, Kwok-Kin Wong, Dana-Farber Cancer Institute, 450 Brookline Avenue HIM 243, Boston, MA 02215, kwong1@partners.org.

*These authors contributed equally

**These authors contributed equally

AUTHOR CONTRIBUTIONS

All authors contributed extensively to the work presented in this paper. E.M.V., H.G.G., and Y.L. contributed equally to this work. K.W. and D.A.B. contributed equally to this work. E.M.V., A.T., A.C., S.L.C., and G.G. contributed computational analyses. E.M.V., M.H., and L.A.G. contributed to sequencing. E.M.V., M.G., C.L., S.M., A.G.G., J.A.W., A.R.R., J.H., G.O., P.J., F.S.H., and D.A.B. contributed clinical input for the patient case and for identifying other relevant cases. V.R., L.S., N.L., S.R. contributed pathology assessments and Profile queries. H.G.G., Y.L., S.K., K.W., T.T., P.S.H., G.D., K.W., and D.A.B. contributed the experimental evaluation of *JAK3* in tumor and immune cells. All authors discussed the results and implications, and commented on the manuscript at all stages.

deregulate cytokine receptor signal transduction could contribute to PD-L1 activation and engagement of the PD-1 immune checkpoint in lung cancer.

INTRODUCTION

The Programmed cell death-1 (PD-1) protein is a co-inhibitory receptor that restrains immune signaling by inhibiting T cell function. Tumors that express its major inducible ligand, PD-L1, evade immunosurveillance by engaging the PD-1 immune checkpoint (1, 2). In preclinical models, blockade of PD-L1 interaction with PD-1 promotes immune-mediated antitumor activity (3). Clinical trials of PD-1 and PD-L1 inhibitors have uncovered durable tumor regression in a subset of patients with a variety of aggressive cancers (4–7). Although studies have suggested that tumors PD-L1 expression in tumors or tumor infiltrating immune cells (8) appear more likely to respond to immune checkpoint inhibition, the specific determinants of this enhanced responsiveness remain incompletely characterized.

Identifying genomic mechanisms of inhibitor sensitivity may inform patient selection for agents targeting immune checkpoints and suggest approaches to enhance their efficacy in otherwise resistant patients. Comprehensive genomic profiling of exceptional responders has revealed the genomic mechanism of extraordinary response to targeted therapies (9–13), but has not yet been applied to immunotherapies.

We identified a patient with advanced, chemorefractory stage IV lung adenocarcinoma who achieved a long-term durable response on a phase I clinical trial of MPDL3280A, an engineered anti-PDL1 antibody (8). To identify genomic mechanisms associated with this sensitivity, we performed genomic profiling of the patient's tumor and germline samples.

Case Report

A 57 year-old male with a 40 pack-year smoking history presented with left shoulder discomfort. Magnetic resonance imaging (MRI) revealed a $1 \times 1.4 \times 2$ cm lytic abnormality in the left humeral head. Biopsy of this lesion identified CK7 and TTF-1 positive adenocarcinoma, suggestive of primary lung origin. Chest computed tomography (CT) demonstrated a $4 \times 3.3 \times 2$ cm left apical mass. Positron emission tomography (PET) confirmed that this mass, the left humeral lesion, and left paratracheal lymphadenopathy were FDG-avid, Brain MRI revealed four lesions consistent with additional metastatic spread.

The patient received palliative whole brain and left shoulder radiotherapy, followed by a single cycle of carboplatin and paclitaxel, which he tolerated poorly (Fig. 1A). He then responded to dose-reduced carboplatin, pemetrexed, and bevacizumab for three additional cycles, and was transitioned to maintenance pemetrexed and bevacizumab.

After 8 months of maintenance therapy, CT scans demonstrated growth of a left adrenal mass. Laparoscopic left adrenalectomy was performed for palliation of severe flank pain and for further molecular profiling. Clinical testing for oncogenic alterations revealed wild-type *EGFR*, *ALK*, and *KRAS* status. Three months later the patient developed a new right adrenal mass and recurrence of the left paratracheal lymphadenopathy. Hospice was considered in

the setting of worsening pain and weight loss (Fig. 1B). Immunohistochemistry (IHC) performed on the excised left adrenal tumor demonstrated PD-L1 reactivity, prompting enrollment on Dana-Farber/Harvard Cancer Center (DF/HCC) clinical trial 11–314, a Phase I study of MPDL3280A, an engineered anti-PD-L1 antibody. The patient received sixteen infusions of MPDL3280A over a one-year period per the specifications of the clinical protocol. He achieved a partial response by RECIST criteria (Fig. 1C), yet more significantly, he experienced complete resolution of his symptoms, discontinued all pain medication, and returned to his pre-diagnosis body weight. He completed one year of therapy per protocol and remained without evidence of disease progression for an additional 12 months. At this point, he began to lose weight again and developed regrowth of the right adrenal mass (Fig. 1D), leading to re-initiation of MPDL3280A therapy. Restaging scans after another 3 months of MPDL3280A showed rapid improvement of the right adrenal lesion (Fig. 1D).

Given the patient's extraordinary and repeated response to PD-L1 immune checkpoint blockade, comprehensive genomic profiling of the patient's tumor and germline DNA was performed.

MATERIALS AND METHODS

Patient informed consenting and tumor/blood samples

All patients provided written informed consent for research biopsies and genomic profiling of tumor and normal DNA, as approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board (DF/HCC Protocol 11–104). Additionally, the patient provided written informed consent to participate in a clinical trial of MPDL3280A (11–314; NCT# 01375842). A blood sample was obtained from the patient for germline testing after the complete response was identified, and whole blood was stored at -80°C until DNA extraction was performed. Tumor specimens for PD-L1 immunohistochemistry and blood samples were obtained from additional patients consented through DF/HCC Protocol 11–104. Normal blood donor samples were accessed through DF/HCC Protocol 10–145.

Histology and Staining

Histopathologic analysis of the surgically resected left adrenal gland revealed metastatic adenocarcinoma, consistent with metastasis from the patient's lung primary. Surgical resection margins were negative for tumor and the tumor was confined by the adrenal capsule. Immunohistochemistry (IHC) for tumor PD-L1 expression was performed as described (14–16). In brief, PD-L1 (9A11, 1:125) was performed on the Leica BOND III platform using Bond's Polymer Refine Detection kit. Heat-induced antigen retrieval was performed in Bond ER2 solution for 30 minutes online. Sections were incubated at room temperature in primary antibody for 120 minutes at room temperature. Upon staining completion, slides were dehydrated and coverslipped offline. S.R. performed all IHC interpretation and was blinded to JAK3 V722I or P132T status when assessing PD-L1 stain. A randomly control set of 9 lung cancers identified by an independent pathologist was stained in parallel to determine relative enrichment. Scoring was performed by measuring the average number of positive cells in a given sample and the average intensity of staining

(0 = no staining, 1+ = weak, 2+ = moderate, 3+ = intense positive staining, with all positive staining considered over background). Determination of statistically significant differences between the groups was performed by calculating an adjusted expression (H) score (% positive cells × staining intensity) (14, 17). Macrophage cells were identified through morphologic determination in intratumoral or alveolar spaces.

Targeted sequencing

Standard techniques were utilized to extract genomic DNA from tumor within the left adrenalectomy specimen and from blood. Initial sequencing of tumor DNA was performed using the OncoMap assay, which detects mutations in 41 cancer genes at 471 different loci using multiplex PCR to amplify the region containing the variant of interest (18). Following primer extension of the allele-specific DNA products, DNA analyses were measured using chip-based mass spectrometry (Sequenom MassARRAY 4). Additional tumor samples that harbored variants of interest in JAK3 were identified through the Dana-Farber Cancer Institute (DFCI) PROFILE project using the OncoMap assay, which included V722I and P132T variants. All lung cancer tumor samples from patients who provided written informed consent (DF/HCC Protocol 11–104) with adequate tumor tissue who underwent OncoMap assay testing done between 2010 and 2013 were included in the query. The OncDRS clinical-genomics database system that links genomic data from PROFILE with salient clinical annotations was utilized to provide a listing of patients with lung cancers who harbored *JAK3* variants of interest, and all such patients with available tumor samples were studied with immunohistochemistry for PD-L1 as described above. These samples were then obtained for additional analyses, including histology and staining for PD-L1 described above.

Whole exome sequencing

Library construction and sequencing—Whole exome capture was performed using the Agilent SureSelect Human All Exon 44Mb v2.0 bait set (Agilent Technologies, USA). In summary genomic DNA was sheared, end repaired, ligated with barcoded Illumina sequencing adapters, amplified, size selected and subjected to in solution hybrid capture using the Agilent SureSelect Human All Exon v2.0 bait set (19, 20). Resulting exome Illumina sequencing libraries were then qPCR quantified, pooled, and sequenced with 76 base paired-end reads using HiSeq2500 sequencers (Illumina, USA). Raw BAM files are deposited in phs000694.v1.p1.

Sequence data processing and quality control—Exome sequence data processing was performed using established analytical pipelines at the Broad Institute. Tumor and normal sequences were aligned to the hg19 human genome build from Illumina sequencing reads using the Picard pipeline (<http://picard.sourceforge.net/>). The BAM was uploaded into Firehose (<http://www.broadinstitute.org/cancer/cga/Firehose>), which manages input and output files. Comparison of the origin for tumor and normal genotypes was performed to assess fingerprinting concordance, and cross-contamination of samples was estimated using ContEst (21).

Alteration identification—MuTect (22) was applied to identify somatic single-nucleotide variants. DNA oxidation artifacts induced during sequencing were computationally removed using a filter-based method(23). Indelocator (<http://www.broadinstitute.org/cancer/cga/indelocator>) was applied to identify small insertions or deletions. Annotation of identified variants was done using Oncotator (<http://www.broadinstitute.org/cancer/cga/oncotator>). Copy ratios were calculated for each captured target by dividing the tumor coverage by the median coverage obtained in a set of reference normal samples. The resulting copy ratios were segmented using the circular binary segmentation algorithm. Genes in copy ratio regions with segment means of greater than $\log_2(4)$ were evaluated for focal amplifications, and genes in regions with segment means of less than $\log_2(0.5)$ were evaluated for deletions. Genome wide copy-ratios were estimated from whole-exome sequencing (WES) data by comparison of the observed depth of coverage at each exon to that achieved in normal samples. Allelic copy-ratios were then estimated by analysis of allelic fractions for all heterozygous SNPs identified in the paired normal sample. Purity and ploidy evaluations to derive absolute copy number were made using ABSOLUTE (24). Heuristic analysis of all somatic alterations was performed using PHIAL (25). Somatic alterations were manually reviewed using Integrated Genomics Viewer (26, 27).

Experimental procedures

Cell culture—293T and Calu-1 cells were maintained in DMEM and RPMI 1640 respectively, with 10% FBS. Beas-2B was maintained in Keratinocyte SFM, supplemented with human recombinant EGF and BPE (Gibco). All media were supplemented with 1% Penicillin/Streptomycin.

Plasmids, immunoblotting, flow cytometry—JAK3 mutant alleles were generated from pDONR223-JAK3-WT (Broad Institute, TRC) using the Quikchange® Lightning Site-Directed Mutagenesis Kit (Agilent Technologies), and transferred into the pLX304 vector using the Gateway LR Clonase II enzyme mix from Life Technologies. 293T cells were transfected using X-tremeGENE HP DNA Transfection Reagent (Roche) with pLX304 EGFP, JAK3-WT or JAK3 constructs as described (28). Lysates were harvested after 48 h and immunoblotting was also performed according to a standard protocol(28). JAK3 (#8827) and Y980/981 pJAK3 (#5031) antibodies were from Cell Signaling Technologies. Beas-2B and Calu-1 cells were infected with lentivirus generated from pLX304 empty control or the same JAK3 constructs and selected in blasticidin to derive stably infected cell lines as described (28). Flow cytometry for PD-L1 expression was performed 72 h after plating, as described in (29). In brief, cells were stained with an anti-PD-L1 antibody (29E. 2A3) or isotype control antibody, and levels of PD-L1 expression were quantified using a BD FACSCanto II® flow cytometer equipped with Diva software (BD Biosciences). Levels were compared with isotype control antibodies. PD-L1 mean fluorescence intensity (MFI) was normalized to isotype control. For EGF stimulation cells were incubated with EGF (50 ng/ml) for 72 h prior to FACS analysis.

PBMC isolation and stimulation—Peripheral blood mononuclear cells (PBMCs) from patients identified as having JAK3 V722I or P132T variants (see “Targeted Sequencing” section above) and healthy donors were isolated from fresh blood and platelet-depleted

blood collars, respectively, by Ficoll method. PBMCs were plated at a density of 7.5×10^5 cells/ml in a 48-well plate and stimulated with 250ng/ml IFN γ (PBL Interferon Source). No stimulation controls were set-up for each donor. At 48 h post-stimulation, cells were treated with 2mM EDTA and collected for flow cytometry to assess for PD-L1 expression on CD14 $^{+}$ myeloid cells. Cells were stained with Live/Dead yellow viability dye (Invitrogen), as well as antibodies (BD Biosciences) against CD14 (M5E2), CD11b (ICRF44), and PD-L1 (MIH1) for 30 min at 4°C, and then fixed in BD Cytfix buffer prior to analyses on BD LSR Fortessa SORT HTS flow cytometer. Given the need to compare the difference between two means in relation to the variation in the data, a t-test was used to compare PD-L1 inducibility between V722I and non-V722I monocytes.

T cell proliferation assay—PBMCs were isolated from patient blood samples immediately pre- and 1 h post-treatment with MPDL3280A, as well as from a normal donor by Ficoll and plated in a density of 4×10^6 cells/ml to a 96-well plate. After 2 h at 37°C non-adherent cells (lymphocyte portion) were removed by pipetting and remaining adherent cells (monocyte portion) were cultured with or without 250ng/ml IFN γ . At 24 h, non-adherent lymphocytes were labeled with CFSE; monocytes (IFN γ treated or untreated) were harvested by 5mM EDTA treatment and resuspended in fresh medium. Both lymphocytes and monocytes were then plated to a 96-well plate pre-coated with 10 μ g/ml OKT3 antibody. Proliferation of CD4 $^{+}$ T cells and CD8 $^{+}$ T cells were monitored at 72 h post-stimulation by CFSE dilution.

RESULTS

Genomic profiling identifies selection for two JAK3 mutations in the tumor

The only variant observed in the mass spectrometric genotyping panel was *JAK3*^{V722I}. This variant, which is located in the pseudokinase or JH2 domain of JAK3 (Fig. 2A, S1A), has been described and functionally characterized as an activating allele in patients with acute megakaryocytic leukemia (30), acute lymphoblastic leukemia (31), and extranodal nasal-type natural killer cell lymphoma (32). *JAK3*^{V722I} has also been identified in peripheral blood from normal subjects (33), and the population frequency of this hyperactive germline variant is approximately 1% (34).

Whereas tumor WES revealed neutral copy of the *JAK3* locus on chromosome 19 in aggregate (Fig. 2B, S2), allele-specific copy number segmentation demonstrated near complete conversion to the mutant allele in this region (Fig. 2C, S3). The allelic fraction of the *JAK3*^{V722I} locus was 0.88 (131/149 reads) in the tumor and 0.47 (90/190 reads) in the germline sample, consistent with homozygosity of the *JAK3*^{V722I} allele in the tumor sample (which was 76% pure) and similar to the selection that occurs for activating *JAK2*^{V617F} alleles in myeloproliferative neoplasms (MPNs) (35).

Next, the 1,767 non-synonymous somatic alterations observed in the WES data were ranked for clinical and biological relevance (Fig. S4, Table S1) (25). Among the clinically relevant events, a second somatic *JAK3* missense mutation at codon 61 (S->C) was observed and orthogonally validated in tumor DNA with PCR (Fig. 2A, S1B). This mutation occurred in the FERM domain and has not been described previously. Since we identified two distinct

genomic events in *JAK3* undergoing tumor somatic selection in cis, a scenario described in hematologic malignancies (36), and Epstein-Barr Virus (EBV) induces PD-L1 expression via *JAK3* (37), we sought to determine whether these *JAK3* mutations were activating and might contribute to PD-L1 mediated immune checkpoint evasion in lung cancer.

Molecular basis of *JAK3* auto-activation and *PD-L1* overexpression

We generated constructs that express *JAK3*^{WT}, *JAK3*^{S61C}, *JAK3*^{V722I}, *JAK3*^{S61C/V722I}, and compared activity with an additional known activating JH2-domain mutation (*JAK3*^{R657Q}) (28), identified as a somatic mutation in squamous cell lung cancer (38). Consistent with the known impact of JH2 domain mutation on relieving *JAK3* autoinhibition (30), transfection of 293T cells revealed that *JAK3*^{V722I}, *JAK3*^{S61C/V722I} or *JAK3*^{R657Q} overexpression resulted in increased *JAK3* autophosphorylation and autoactivation compared with the wild-type control as measured by immunoblotting (Fig. 2D). Although levels of *JAK3*^{S61C} phosphorylation did not differ significantly from *JAK3*^{WT}, expression of *JAK3*^{S61C/V722I} caused the highest levels of *JAK3* phosphorylation among all mutants, consistent with the positive selection observed in the tumor and cooperative gain of function.

We next examined the consequences of stable *JAK3* transduction on PD-L1 cell surface expression in immortalized lung epithelial cells (BEAS-2B) and lung cancer cells (Calu-1) (Fig. 3A). Low-level *JAK3*^{S61C/V722I} expression in BEAS-2B cells modestly induced surface PD-L1 by flow cytometry relative to control, as compared to no induction at all in *JAK3*^{WT} expressing cells (Fig. 3B). In contrast, 5-fold greater expression of *JAK3* in Calu-1 increased PD-L1 levels more demonstrably, irrespective of the allele (Fig. 3B). We also examined the consequence of exposure to factors in the lung tumor microenvironment such as EGF, since activation of EGFR signaling known to enhance PD-L1 expression in lung cancer (17, 29). Levels of PD-L1 in Calu-1 cells expressing *JAK3*^{S61C/V722I} were as high as control cells stimulated with EGF, and we observed an additive further increase of PD-L1 expression in mutant cells upon EGF exposure (Fig. 3C). These findings reveal that *JAK3* activation in lung airway and cancer cells induces PD-L1, including wild type kinase when overexpressed at high levels. Furthermore, activated *JAK3* cooperates with factors such as EGF to boost PD-L1 expression even further.

Dual impact on the tumor and immune microenvironment

Consistent with these results, IHC of the patient's tumor using a validated antibody (16) revealed strong positive membrane PD-L1 expression on both tumor and immune cells (macrophages), coupled with increased nuclear pSTAT3 staining, a marker of JAK pathway activation (Fig. 4A). To assess the generalizability of this relationship, we identified 10 out of 500 lung adenocarcinoma patients (2%) previously genotyped for the *JAK3*^{V722I} mutation at our institution (39), including this index case. PD-L1 positivity was observed in tumor cells and more strikingly in macrophages in 9/10 *JAK3*^{V722I} mutated cases (Fig. 4B). PD-L1 positivity was substantially enriched as compared to a random control set of lung cancers (tumor cells: $p = 0.02$; immune cells: $p < 0.01$; Mann-Whitney; Fig. S5), including 4 patients carrying the *JAK3*^{P132T} variant, with the exception of high level PD-L1 tumor expression associated with an ALK rearrangement and another tumor with high level PD-L1 expression in macrophages (Figure 4B).

Because of the strong activation in the immune compartment, and the presence of *JAK3*^{V722I} in the germline, we also measured the inducibility of PD-L1 expression in available matched patient PBMCs. Stimulation with IFN- γ , another cytokine known to trigger PD-L1 in the tumor immune microenvironment, resulted in modest but significantly increased expression of PD-L1 on CD14⁺ myeloid cells from *JAK3*^{V722I} positive patients compared to a *JAK3*^{P132T} positive patient or negative blood donor controls (Fig. 4C). Next, to determine if this increased PD-L1 expression directly inhibits T cells, we collected blood from the index patient immediately pre- and 1 h post- MPDL3280A infusion and exposed monocytes from these samples to the patient's own activated T cells, or from allogeneic T cells from a different donor. In both instances we found that T cell activation was significantly greater in the presence of circulating MPDL3280A, especially when monocytes were primed with IFN γ (Fig. 4D, S6). This enhanced T cell activity correlated with the clinical response we observed upon MPDL3280A rechallenge (Fig. 1D). Thus, monocytes/macrophages that carry the *JAK3*^{V722I} allele also express increased levels of PD-L1, with can contribute directly to T-cell suppression.

Taken together, these findings suggest that, in addition to somatic alteration in lung cancer cells, germline expression of the *JAK3*^{V722I} allele in infiltrating immune cells may represent a key contributor of PD-L1 tumor immune checkpoint engagement.

DISCUSSION

Immune targeting of the PD-L1/PD1 interaction is emerging as an effective therapy for multiple aggressive tumor types, including non-small cell lung cancer (5), and results in occasional long-term responses. While tumor or immune cell PD-L1 expression may indicate a suppressed immune microenvironment and enrich for clinical activity (40), the molecular basis and markers of response remain unclear.

We genomically characterized a patient with metastatic lung adenocarcinoma who experienced an exceptional and durable response to PD-L1 inhibition. We discovered one germline *JAK3* variant and one somatic *JAK3* mutation in the patient's tumor in cis, and we demonstrated that these genetic alterations act in concert to activate JAK3. Stable transduction of this double-mutant increased PD-L1 expression in lung cells. Furthermore, the presence of *JAK3*^{V722I} in the germline, the strong tumor immune cell PD-L1 positivity, and the enhanced PD-L1 induction by IFN- γ in monocytes, which inhibits T cell activation in an MPDL3280A sensitive manner, also suggest a more complex interaction with the tumor microenvironment. To our knowledge, this is the first report that illustrates how a genomic mechanism that impacts both tumor cells and the host response may enhance PD-L1 expression and immune evasion by engaging the PD1 immune checkpoint.

Multiple reports have identified candidate mechanisms that may predict response to immune checkpoint blockade. These may include high levels of tumor-specific neoantigens (41, 42) or inherited immune-related characteristics (43). It is unclear yet whether the specific genomic mechanism of *JAK3* activation reported here is generalizable to other patients who achieve such responses, whether this patient's tumor harbored a rare genotype, or whether the genotype in this patient acted in concert with other somatic or germline alterations.

Notably, JAK3 signaling regulates EBV mediated PD-L1 expression in lymphomas (37) and has been implicated in response to PD-1 blockade in Hodgkin's lymphoma (6), STAT3 binds directly to the PD-L1 promoter (44), and other activating mutations such as *JAK3*^{R657Q} are found in lung cancer (38), supporting the potential generalizability of the findings described herein.

In light of our observations that *JAK3*^{WT} overexpression also induced PD-L1, it is notable that the *JAK2* amplicon, which also contains the PD-L1 locus, is recurrently amplified in lymphoma and EBV-positive gastric cancer (45, 46). Thus, functional SNP variants, somatic alterations resulting in activation of other JAK family members (e.g. *JAK1*, *JAK2*, or *TYK2*), or inactivation of negative regulators such as suppressor of cytokine signaling (*SOCS*) family members may serve as a common pathway for upregulating PD-L1 expression and predicting responsiveness to this immune therapy. Indeed, since this particular *JAK3*^{V722I} variant is present in the germline at a significantly lower frequency (1–2%) compared with the frequency of PD-L1 positivity in lung cancer overall (at least 20%), it may only explain a small subset of tumors that engage this pathway. But since long-term durable remissions are much rarer, it is possible that studies in patients enriched for this genotype may show similarly impressive responsiveness as seen in this case. Regardless, further studies in larger patient cohorts are necessary to establish definitively the role of this and other somatic/germline JAK alterations as a mechanism-based predictive marker of response to PD-L1 inhibition.

Broadly, this result expands the concept of studying extraordinary responses to cancer therapeutics beyond classical targeted therapies to include approved or investigational immunotherapies. The identification of these and other genomic mechanisms of sensitivity to immune checkpoint inhibitor blockade will not only help tailor this therapy in a personalized fashion, but may also suggest pharmacologic approaches to induce sensitivity in otherwise resistant patients. Finally, profiling patients who demonstrate initial responses but develop acquired resistance may further illuminate the spectrum of pathways that restrict tumor immunity and implicate additional rational modalities for therapeutic development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors would like to thank the Dana-Farber Cancer Institute Profile project.

Funding: This work was supported by the American Cancer Society (E.M.V.), U.S. NIH R01 CA122794, R01 CA166480, R01 CA163896, R01CA140594, and U01CA141576 (K.W.), K08 CA138918-01A1 (D.A.B), Uniting Against Lung Cancer (K.W. and D.A.B), the GTM Fund for Lung Cancer Research (D.A.B), the Damon Runyon Cancer Research Foundation (P.S.H.)

Conflict of interest disclosures: Dr. Van Allen is a consultant for Synapse and Roche Ventana. Dr. Sholl is a consultant for Genentech. Dr. Oxnard is a consultant for Genentech and AstraZeneca. Dr. Janne is a consultant for Roche and AstraZeneca. Dr. Hammerman is a consultant for Janssen Oncology. Dr. Garraway is an equity holder and consultant in Foundation Medicine, and is consultant to Novartis, Millenium/Takeda, and Boehringer Ingelheim, and a recipient of a grant from Novartis. Dr. Hodi has a commercial research grant from Bristol-Meyers Squibb, institutional clinical trial support from Genentech, Bristol-Meyers Squibb, and Merck, is a consultant for Bristol-Meyers Squibb, Roche, Merck, and Novartis. Dr. Rodig has a commercial research grant from Bristol-

Meyers-Squibb, is a consultant for AstraZeneca, and has a patent application for use of monoclonal antibodies for PDL1 IHC. Dr. Dranoff has commercial research grants from Novartis and Bristol-Meyers-Squibb and is a consultant for Novartis. Dr. Barbie is a consultant for N-of-One.

REFERENCES

1. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nature medicine*. 2002; 8(8): 793–800. PubMed PMID: 12091876.
2. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *The Journal of experimental medicine*. 2000; 192(7):1027–1034. PubMed PMID: 11015443; PubMed Central PMCID: PMC2193311. [PubMed: 11015443]
3. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99(19):12293–12297. PubMed PMID: 12218188; PubMed Central PMCID: PMC129438. [PubMed: 12218188]
4. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *The New England journal of medicine*. 2012; 366(26):2455–2465. PubMed PMID: 22658128; PubMed Central PMCID: PMC3563263. [PubMed: 22658128]
5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *The New England journal of medicine*. 2012; 366(26):2443–2454. PubMed PMID: 22658127; PubMed Central PMCID: PMC3544539. [PubMed: 22658127]
6. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 Blockade with Nivolumab in Relapsed or Refractory Hodgkin's Lymphoma. *The New England journal of medicine*. 2014 PubMed PMID: 25482239.
7. Powles T, Eder JP, Fine GD, Braiteh FS, Loria Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014; 515(7528):558–562. PubMed PMID: 25428503. [PubMed: 25428503]
8. Herbst RS, Soria JC, Kowanzet M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014; 515(7528): 563–567. PubMed PMID: 25428504. [PubMed: 25428504]
9. Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, et al. Genome sequencing identifies a basis for everolimus sensitivity. *Science*. 2012; 338(6104):221. Epub 2012/08/28. PubMed PMID: 22923433. [PubMed: 22923433]
10. Al-Ahmadie H, Iyer G, Hohl M, Asthana S, Inagaki A, Schultz N, et al. Synthetic lethality in ATM-deficient RAD50-mutant tumors underlies outlier response to cancer therapy. *Cancer discovery*. 2014; 4(9):1014–1021. PubMed PMID: 24934408; PubMed Central PMCID: PMC4155059. [PubMed: 24934408]
11. Imielinski M, Greulich H, Kaplan B, Araujo L, Amann J, Horn L, et al. Oncogenic and sorafenib-sensitive ARAF mutations in lung adenocarcinoma. *The Journal of clinical investigation*. 2014; 124(4):1582–1586. PubMed PMID: 24569458; PubMed Central PMCID: PMC3973082. [PubMed: 24569458]
12. Wagle N, Grabiner BC, Van Allen EM, Amin-Mansour A, Taylor-Weiner A, Rosenberg M, et al. Response and acquired resistance to everolimus in anaplastic thyroid cancer. *The New England journal of medicine*. 2014; 371(15):1426–1433. PubMed PMID: 25295501. [PubMed: 25295501]
13. Wagle N, Grabiner BC, Van Allen EM, Hodis E, Jacobus S, Supko JG, et al. Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. *Cancer discovery*. 2014; 4(5):546–553. PubMed PMID: 24625776; PubMed Central PMCID: PMC4122326. [PubMed: 24625776]
14. Choueiri TK, Fay AP, Gray KP, Callea M, Ho TH, Albiges L, et al. PD-L1 expression in nonclear-cell renal cell carcinoma. *Annals of oncology : official journal of the European Society for*

Medical Oncology / ESMO. 2014; 25(11):2178–2184. PubMed PMID: 25193987. [PubMed: 25193987]

15. Shi M, Roemer MG, Chapuy B, Liao X, Sun H, Pinkus GS, et al. Expression of programmed cell death 1 ligand 2 (PD-L2) is a distinguishing feature of primary mediastinal (thymic) large B-cell lymphoma and associated with PDCD1LG2 copy gain. *The American journal of surgical pathology*. 2014; 38(12):1715–1723. PubMed PMID: 25025450. [PubMed: 25025450]
16. Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2013; 19(13):3462–3473. PubMed PMID: 23674495. [PubMed: 23674495]
17. Azuma K, Ota K, Kawahara A, Hattori S, Iwama E, Harada T, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2014; 25(10):1935–1940. PubMed PMID: 25009014. [PubMed: 25009014]
18. MacConaill LE, Campbell CD, Kehoe SM, Bass AJ, Hatton C, Niu L, et al. Profiling critical cancer gene mutations in clinical tumor samples. *PloS one*. 2009; 4(11):e7887. Epub 2009/11/20. PubMed PMID: 19924296; PubMed Central PMCID: PMC2774511. [PubMed: 19924296]
19. Fisher S, Barry A, Abreu J, Minie B, Nolan J, Delorey TM, et al. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. *Genome biology*. 2011; 12(1):R1. Epub 2011/01/06. PubMed PMID: 21205303; PubMed Central PMCID: PMC3091298. [PubMed: 21205303]
20. Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, Brockman W, et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nature biotechnology*. 2009; 27(2):182–189. Epub 2009/02/03. PubMed PMID: 19182786; PubMed Central PMCID: PMC2663421.
21. Cibulskis K, McKenna A, Fennell T, Banks E, DePristo M, Getz G. ContEst: estimating cross-contamination of human samples in next-generation sequencing data. *Bioinformatics*. 2011; 27(18):2601–2602. Epub 2011/08/02. PubMed PMID: 21803805; PubMed Central PMCID: PMC3167057. [PubMed: 21803805]
22. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature biotechnology*. 2013 Epub 2013/02/12. PubMed PMID: 23396013.
23. Costello M, Pugh TJ, Fennell TJ, Stewart C, Lichtenstein L, Meldrim JC, et al. Discovery and characterization of artifactual mutations in deep coverage targeted capture sequencing data due to oxidative DNA damage during sample preparation. *Nucleic acids research*. 2013; 41(6):e67. Epub 2013/01/11. PubMed PMID: 23303777; PubMed Central PMCID: PMC3616734. [PubMed: 23303777]
24. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nature biotechnology*. 2012; 30(5):413–421. Epub 2012/05/01. PubMed PMID: 22544022.
25. Van Allen EM, Wagle N, Stojanov P, Perrin DL, Cibulskis K, Marlow S, et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. *Nature medicine*. 2014; 20(6):682–688. PubMed PMID: 24836576; PubMed Central PMCID: PMC4048335.
26. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. *Nature biotechnology*. 2011; 29(1):24–26. Epub 2011/01/12. PubMed PMID: 21221095; PubMed Central PMCID: PMC3346182.
27. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in bioinformatics*. 2013; 14(2):178–192. Epub 2012/04/21. PubMed PMID: 22517427; PubMed Central PMCID: PMC3603213. [PubMed: 22517427]
28. Zhu Z, Aref AR, Cohoon TJ, Barbie TU, Imamura Y, Yang S, et al. Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. *Cancer discovery*. 2014; 4(4):452–465. PubMed PMID: 24444711; PubMed Central PMCID: PMC3980023. [PubMed: 24444711]

29. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer discovery*. 2013; 3(12):1355–1363. PubMed PMID: 24078774; PubMed Central PMCID: PMC3864135. [PubMed: 24078774]
30. Walters DK, Mercher T, Gu TL, O'Hare T, Tyner JW, Loriaux M, et al. Activating alleles of JAK3 in acute megakaryoblastic leukemia. *Cancer cell*. 2006; 10(1):65–75. PubMed PMID: 16843266. [PubMed: 16843266]
31. Yin C, Sandoval C, Baeg GH. Identification of mutant alleles of JAK3 in pediatric patients with acute lymphoblastic leukemia. *Leukemia & lymphoma*. 2014;1–13. PubMed PMID: 25146434.
32. Boucekhoua A, Scourzic L, de Wever O, Zhang Y, Cervera P, Aline-Fardin A, et al. JAK3 deregulation by activating mutations confers invasive growth advantage in extranodal nasal-type natural killer cell lymphoma. *Leukemia*. 2014; 28(2):338–348. PubMed PMID: 23689514. [PubMed: 23689514]
33. Riera L, Lasorsa E, Bonello L, Sismondi F, Tondat F, Di Bello C, et al. Description of a novel Janus kinase 3 P132A mutation in acute megakaryoblastic leukemia and demonstration of previously reported Janus kinase 3 mutations in normal subjects. *Leukemia & lymphoma*. 2011; 52(9):1742–1750. PubMed PMID: 21599579. [PubMed: 21599579]
34. Exome Variant Server. Available from: URL: <http://evs.gs.washington.edu/EVS/>.
35. Gonzalez MS, De Brasi CD, Bianchini M, Gargallo P, Stanganelli C, Zalcberg I, et al. Improved diagnosis of the transition to JAK2 (V(6)(1)(7)F) homozygosity: the key feature for predicting the evolution of myeloproliferative neoplasms. *PloS one*. 2014; 9(1):e86401. PubMed PMID: 24475114; PubMed Central PMCID: PMC3903535. [PubMed: 24475114]
36. Bergmann AK, Schneppenheim S, Seifert M, Betts MJ, Haake A, Lopez C, et al. Recurrent mutation of JAK3 in T-cell prolymphocytic leukemia. *Genes, chromosomes & cancer*. 2014; 53(4):309–316. PubMed PMID: 24446122. [PubMed: 24446122]
37. Green MR, Rodig S, Juszczynski P, Ouyang J, Sinha P, O'Donnell E, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012; 18(6):1611–1618. PubMed PMID: 22271878; PubMed Central PMCID: PMC3321508. [PubMed: 22271878]
38. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. *Nature*. 2012; 489(7417):519–525. PubMed PMID: 22960745; PubMed Central PMCID: PMC3466113. [PubMed: 22960745]
39. MacConaill LE, Garcia E, Shivdasani P, Ducar M, Adusumilli R, Breneiser M, et al. Prospective Enterprise-Level Molecular Genotyping of a Cohort of Cancer Patients. *The Journal of molecular diagnostics : JMD*. 2014 PubMed PMID: 25157968.
40. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti-PD-1 Therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2014 PubMed PMID: 24714771.
41. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome research*. 2014; 24(5):743–750. PubMed PMID: 24782321; PubMed Central PMCID: PMC4009604. [PubMed: 24782321]
42. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *The New England journal of medicine*. 2014; 371(23):2189–2199. PubMed PMID: 25409260. [PubMed: 25409260]
43. Breunis WB, Tarazona-Santos E, Chen R, Kiley M, Rosenberg SA, Chanock SJ. Influence of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) common polymorphisms on outcome in treatment of melanoma patients with CTLA-4 blockade. *Journal of immunotherapy*. 2008; 31(6): 586–590. PubMed PMID: 18528295; PubMed Central PMCID: PMC3276400. [PubMed: 18528295]
44. Wolfe SJ, Strebovsky J, Bartz H, Sahr A, Arnold C, Kaiser C, et al. PD-L1 expression on tolerogenic APCs is controlled by STAT-3. *European journal of immunology*. 2011; 41(2):413–424. PubMed PMID: 21268011. [PubMed: 21268011]

45. Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood*. 2010; 116(17):3268–3277. PubMed PMID: 20628145; PubMed Central PMCID: PMC2995356. [PubMed: 20628145]
46. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014 Epub 2014/08/01. PubMed PMID: 25079317.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

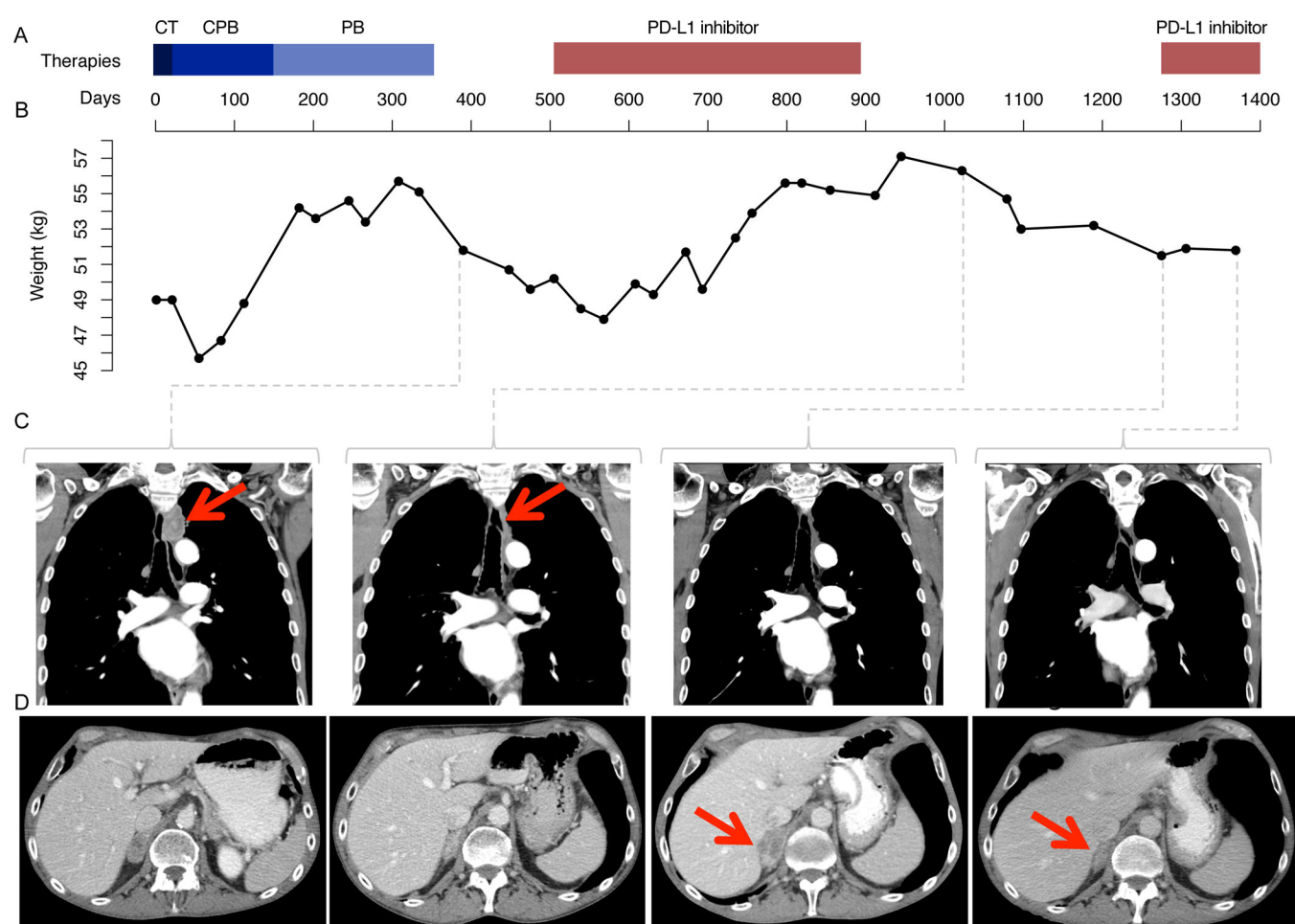


Figure 1. Long-term durable response to PD-L1 blockade in a patient with metastatic lung adenocarcinoma

A. Systemic therapies received over time. CT = carboplatin/paclitaxel, CPB = carboplatin/pemetrexed/bevacizumab, PB = maintenance pemetrexed/bevacizumab, PD-L1 inhibitor = MPDL3280A. **B.** Change in patient weight (kg) during the same time period. **C.** Serial chest CT scans showing reduction in size of the paratracheal mass over time (arrows). **D.** Serial abdominal CT scans showing recurrence and re-treatment response of the right adrenal mass (arrows).

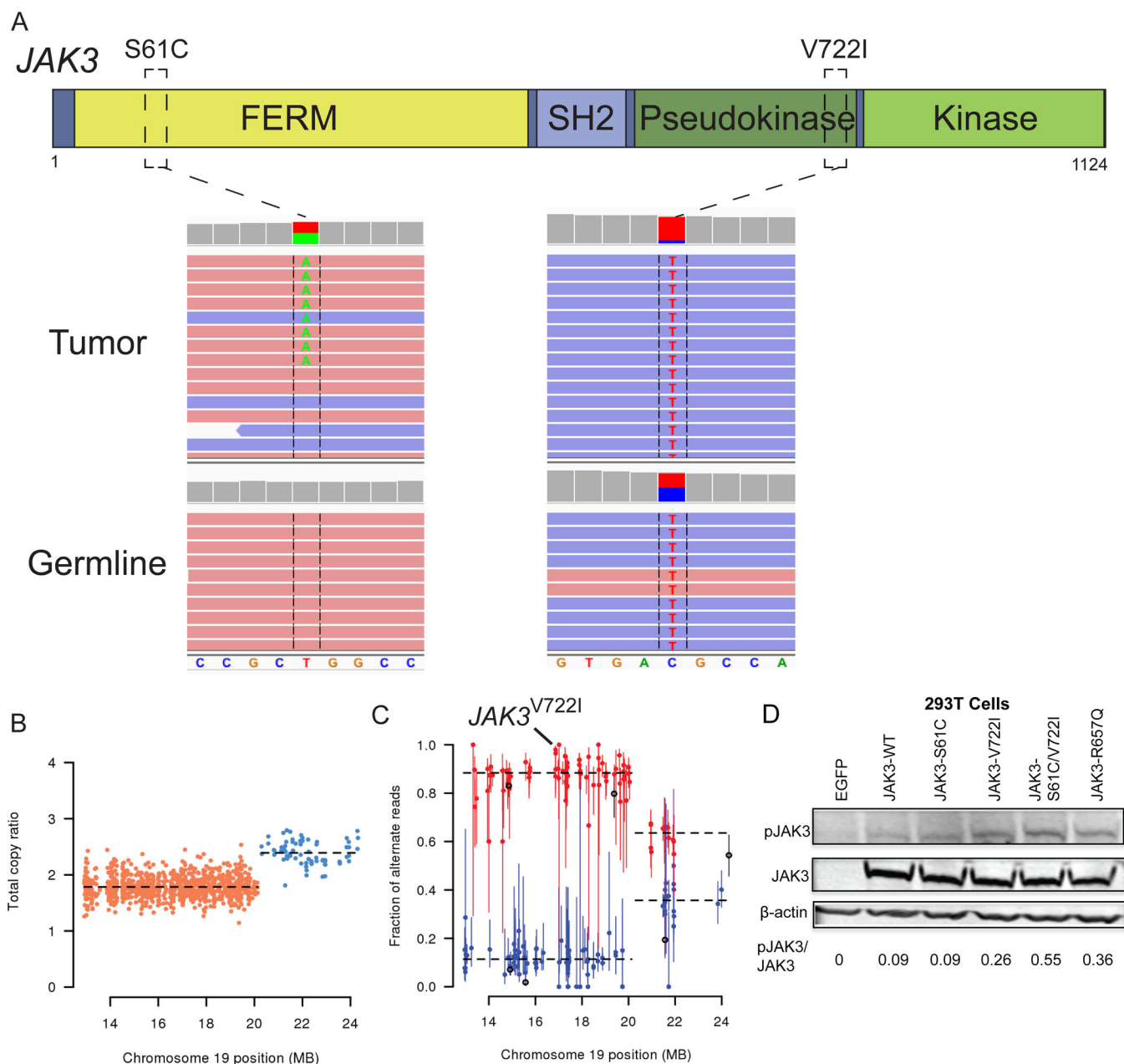


Figure 2. Genomic profiling identifies two *JAK3* alterations present in the tumor that result in constitutive *JAK3* activation

A. Structural organization of *JAK3* including the N-terminal FERM domain, the SH2 domain, and the JH2 or pseudokinase domain, which is adjacent to the kinase domain and contributes to autoinhibition. Sequencing of position 722 of *JAK3* in the JH2 domain reveals heterozygosity for alleles in the germline consistent with a single copy of *JAK3*^{V722I}, while the left adrenal metastasis revealed loss of heterozygosity (LOH) and complete acquisition of the *JAK3*^{V722I} allele (predominant red over blue coverage band). The somatic *JAK3*^{S61C} mutation is also observed using the Integrated Genomics Viewer (IGV). **B.** Whole exome sequencing data revealed apparent copy number neutrality of the *JAK3* locus on chromosome 19. **C.** When analyzed at the allelic level clonality of the *JAK3*^{V722I} allele was

detected, indicated in red, consistent with the focused sequencing results. **D.** Immunoblot of total JAK3, and tyrosine phosphorylated (Y980/981) pJAK3 in 293T cells transfected with EGFP control vector, *JAK3^{WT}*, *JAK3^{S61C}*, *JAK3^{V722I}*, *JAK3^{S61C/V722I}* or *JAK3^{R657Q}*.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

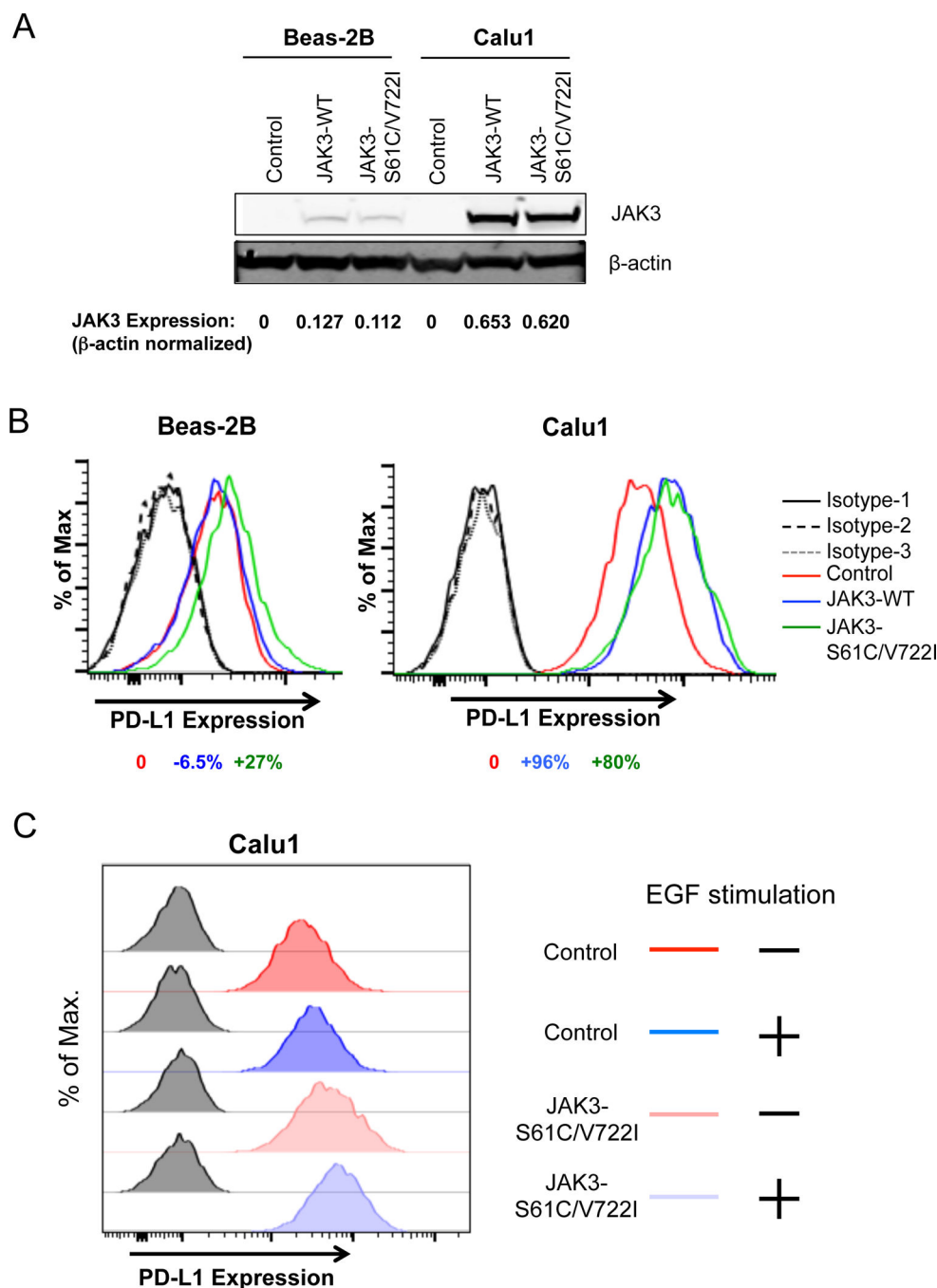


Figure 3. Deregulated JAK3 signaling induces PD-L1 expression in lung cells

A. Immunoblot of total JAK3 levels following stable transduction of *JAK3*^{WT} or the patient derived *JAK3*^{S61C/V722I} alleles in BEAS-2B or Calu-1 cells. **B.** Levels of cell surface PD-L1 expression on these same BEAS-2B or Calu-1 cells were measured by flow cytometry using a PD-L1 specific monoclonal antibody compared to isotype. Highlighted is percent (change in isotype-normalized mean fluorescence intensity (MFI) relative to control). **C.** Cell surface PD-L1 expression on Calu-1 cells expressing control vector or the patient derived *JAK3*^{S61C/V722I} allele, stimulated with or without EGF.

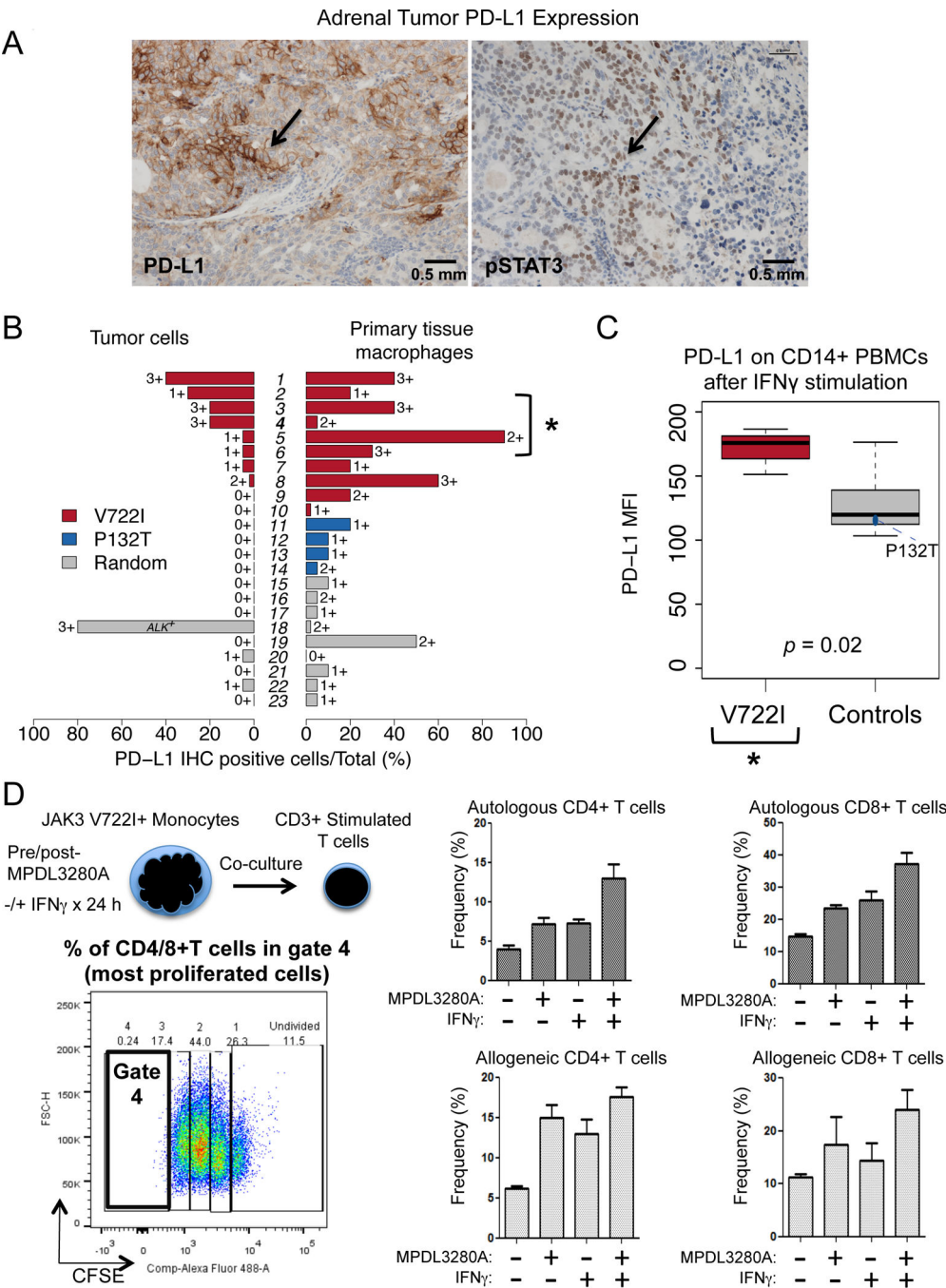


Figure 4. Germline contribution of *JAK3*^{V722I} to immune cell PD-L1 expression and T cell suppression

A. PD-L1 and pSTAT3 immunohistochemistry of the patient's adrenal metastasis (arrows denote example tumor cells). **B.** Levels of tumor cell or immune cell PD-L1 positivity by immunohistochemistry (IHC) across a panel of thoracic malignancies including *JAK3*^{V722I} and *JAK3*^{P132T} positive cases or *JAK3*^{WT} controls (% positive cells and staining intensity, from 0 to 3+, is listed for each tumor and immune cell population from each sample). The case report patient (#4) is marked in bold. **C.** PD-L1 MFI on CD14+ myeloid cells from two

patients (corresponding to patients 2, 3 and 4 in 3C, denoted with asterisk) or donor PBMCs (n = 14) stimulated with IFN-gamma for 48 hours ($p = 0.02$; t-test). **D.** Blood samples were drawn from the index patient immediately pre- and 1 h post- MPDL3280A infusion, and monocytes $-/+$ IFN γ stimulation were incubated with T cells from the patient (autologous, pre-MPDL3280A) or a donor (allogeneic). T cell proliferation (frequency of positive cells in gate 4) is shown for autologous or allogeneic CD4 $^{+}$ or CD8 $^{+}$ T cells under each condition.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript