**METHODS
Study design and patient characteristics**

All 3 clinical studies were nonrandomized, open-label trials in patients with nonsquamous NSCLC designed to evaluate the maximum tolerated dose (MTD) or optimal biological dose (OBD) of study drugs, with secondary objectives of assessing safety, pharmacokinetics, immunogenicity, and antitumor activity. Study 1108 was a basket-style study enrolling patients with different malignant solid tumors, including NSCLC, who had failed previous chemotherapy or for whom no standard therapy was available. Patients received durvalumab 10 mg/kg Q2W. ATLANTIC and Study 006 enrolled patients with locally advanced or metastatic NSCLC (Stage IIIB-IV) who had received at least two prior systemic treatment regimens including one platinum-based chemotherapy regimen. Patients in ATLANTIC received durvalumab 10 mg/kg Q2W and those in Study 006 received durvalumab 20 mg/kg plus tremelimumab 1 mg/kg Q4W. Treatment continued until confirmed progressive disease, initiation of alternative cancer therapy, unacceptable toxicity, or other reasons to discontinue treatment.

**mRNA sequencing on frozen biopsies from clinical specimens**

To summarize, RNA was extracted utilizing the Zymo Quick-RNA MicroPrep according to the manufacturer's protocol and checked for quantity and quality via spectrophotometry and Agilent bioanalyzer. Sufficient quality RNA was converted to libraries utilizing TruSeq RNA chemistry, and libraries were sequenced on the Illumina HiSeq instrument. For all sequencing data, reads were quality checked for read counts, quality values, kmer usage, GC content, and all other relevant parameters with FastQC (v0.10.1) and custom scripts. A minimum read count of 80 million (100 base pairs, paired end) was required, and reads were mapped to human reference genome (UCSC hg19; February 2009 release; Genome Reference Consortium GRCh37; gtf annotation file GRCh37.68), using STAR (v2.5.2) with at least 70% mapping rates and were quantified as transcripts per million using RSEM. RNA-sequencing data from Study 1108 have been deposited into the Gene Expression Omnibus (GEO) repository (ID number GSE110390). Gene expression profile was analyzed by using R software. Functional annotations of genes differentially expressed were conducted using Ingenuity Pathway Analysis (IPA).

**DNA sequencing and tumor mutation burden analysis**

Briefly, TMB was defined as the number of somatic, coding base substitutions, and short indels per megabase of genome examined. All base substitutions and indels in the coding region of targeted genes, including synonymous mutations, were filtered for both oncogenic driver events according to COSMIC and germline status according to dbSNP and ExAC databases, in addition to a private database of rare germline events compiled in the Foundation Medicine clinical cohort. Additional filtering based upon a computational assessment of germline status using the SGZ (somatic-germline-zygosity) tool was also performed.

**Flow cytometry-based immunophenotyping on patients’ whole blood**

Fifty μL of whole blood was transferred to 12x75 mm polystyrene tubes to which a cocktail of pre-mixed and optimally diluted fluorochrome-conjugated antibodies had been added. After a 20-minute incubation period at room temperature, an erythrocyte lysing solution was added to each tube and incubated for 5 minutes. Samples were run on Becton Dickinson FACSCanto II instruments with three lasers (405 nm, 488 nm and 633 nm) and the manufacturer’s standard 4-2-2 optical configuration. All populations were reported as both population percentages and absolute counts in cells/mm3. The latter values were calculated by multiplying the population percentage by the absolute count of T cells determined from the T, B, and NK cell assay.

**Western blot assay**

Cell pellets were lysed with cell lysis buffer (Cell Signaling Technology, catalog #9803) containing protease and phosphatase inhibitors and protein concentrations determined. After reconstitution to 1X sample buffer using concentrated 6X SDS Sample Buffer (Boston Bioproduct, catalog #BP-111R), equal amounts of protein per sample were resolved by 4-12% Bis-Tis gel electrophoresis (Thermo Fisher Scientific, catalog #WG1402BOX) and then transferred to PVDF membranes (Thermo Fisher Scientific, catalog #IB24001). The following antibodies were used for blotting: anti*-STK11* (Cell Signaling Technology, catalog #3047; RRID:AB\_2198327), anti-p*STK11* (Cell Signaling Technology, catalog #3482; RRID:AB\_2198321), anti-AMPK (Cell Signaling Technology, catalog #5831; RRID:AB\_10622186), anti-phospho-AMPK (Cell Signaling Technology, catalog #2535; RRID:AB\_331250) and anti-β-actin (Cell Signaling Technology, catalog #5125; RRID:AB\_1903890). HRP conjugated anti-rabbit IgG secondary antibody (GE Healthcare, catalog #NA934; RRID:AB\_772206) was bound to primary Abs and detected using SuperSignal West Pico PLUS Chemiluminescent Substrate enhanced chemiluminescence (Thermo Fisher Scientific, catalog #34578). Images were captured and quantified by ImageQuant software (GE Healthcare, LAS4000mini).

**RNA extraction, library preparation and sequencing on mouse models**

The extracted RNA was recovered in 30 µL and evaluated by Agilent Tape Station 4200 RNA (catalog #5067-5576) to determine quality (RIN scores) and quantified by Qubit using Invitrogen’s RNA BR assay (catalog #Q10211). Library preparation was carried out per manufacturer’s protocol using the reagents provided in the Illumina TruSeq Stranded mRNA Library Prep Kit (catalog #20020594). A total of 2 ng RNA starting material was used for library capture, a process that captures coding transcripts with strand information by converting the mRNA in total RNA into polyadenylated fragmented cDNA, which is then copied into first strand cDNA using reverse transcriptase and random primers, followed by second strand synthesis prior to final purification and PCR enrichment. The final library products were then validated by Agilent Tape Station 4200 DNA 1000 HS (catalog #5067-5584) to determine fragment size and distribution and quantified by qPCR to accurately calculate the of number of amplifiable molecules using KAPA Illumina Library Quantification Kit (catalog #KK4873). Libraries were then normalized to 4 nM for final pooling and denaturing according to manufacturer’s recommendations for paired-end/single-index sequencing on the Illumina NextSeq500 sequencer.

**Mouse tumor models and treatments**

Balb/c mice 6-9 weeks of age were purchased from Envigo Harlan Laboratories, Inc. (Indianapolis, IN). NSG mice used for engraftment of human patient-derived xenografts (PDXs) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were humanely treated and housed according to Institutional Animal Care and Use Committee approved protocols in the Laboratory Animal Resources facility at AstraZeneca, an Association for Animal Accreditation of Laboratory Animal Care and United States Department of Agriculture-licensed facility. PDX tissues were obtained from AstraZeneca’s own internal PDX library. *In vivo* passage number and *STK11* mutational status are shown in the table below:

|  |  |  |
| --- | --- | --- |
| **PDX Tumor Model** | **Passage number** | ***STK11* mutation status** |
| LG0645 | 3 | DeepDel |
| MEDI-NSCLC-03 | 5 | stopgain: K41X |
| MEDI-NSCLC-04 | 5 | stopgain: E256X |
| MEDI-NSCLC-19 | 6 | M129I, Q302K, E130X |
| HLXF-23 | 3 | WT |
| MEDI-NSCLC-15 | 6 | WT |
| HLFX-036LN | 3 | WT |
| HLXF-048 | 2 | WT |
| HLXF-093 | 2 | WT |
| HLXF-16 | 3 | WT |
| LG0567 | 4 | WT |

**Flow cytometry of mouse tumor models**

Tumor sample for flow cytometry were digested with a mix of collagenase and other enzymes and dissociated using a Miltenyi GentleMacs™ Dissociator; spleens were dissociated using mechanical means; TDLNs were digested using a mix of collagenase and other enzymes then heat. Red cell lysis was performed using ACK lysing buffer (Thermo Fisher Scientific) on single-cell suspensions of spleen cells. One million viable cells were plated into individual wells of 96-well u bottom plates. Cells were incubated with fixable blue live/dead dye (Thermo Fisher Scientific) and Fc receptors were blocked using purified anti-mouse CD16/CD32 in flow cytometry buffer (PBS plus 2% fetal bovine serum). Flow antibodies binding to extracellular markers were added and incubated at 4°C for 20 minutes followed by washes in flow cytometry buffer. Cells were then fixed and permeabilized in 1X fix/perm solution (EBioscience) for 30 minutes at 4°C followed by two washes with 1x permeabilization buffer (EBioscience). Cells were then stained for intracellular markers using ICS flow antibodies for 30 minutes at room temperature. Cells were subsequently washed with permeabilization buffer and analyzed using a Symphony Flow Cytometer (BD Biosciences, Franklin Lakes, NJ) and flow cytometry standard (fcs) data analyzed using FlowJo™ software (BD Biosciences, San Jose, CA).

Antibodies used for flow cytometry analysis included:

From BioLegend (San Diego, CA): CD64 Fitc clone X54-5/7.1 (RRID:AB\_2566555), MHCII PeCy7 clone M5/114-15.2 (RRID: AB\_2290801), CD11C BV421 clone BM8 (
AB\_10897814), CD86 APC clone GL1 (RRID:AB\_493343), CD11B BV650 clone M1/70 (RRID:AB\_11125575), Ly6C BV711 clone HK1.4 (RRID:AB\_2562630), CD45 BV785 clone 30-F11 (RRID:AB\_2564590), PD-L1 BV605 clone 10F9G2 (RRID:AB\_2563635), CD25 PerCPcy5.5 clone PC61 (RRID:AB\_893291) and CD69 PE clone H1.2F3 (RRID:AB\_313110).

From Becton Dickinson (Franklin Lakes, NJ): Ly6G BUV395 clone 1A8, B220 BUV661 clone RA36B2, CD24 BUV737 clone MI69, NKP46 BV480 clone 29A1.4, CD4 BUV395 GK1.5, CD8 BUV737 clone 53-6.7, Granzyme b BV421 clone GB11 CD3 BV605 clone 17A2, and IFNg BV711 clone XMG1.2.

From R&D Systems (Minneapolis, MN): Arg1 PE sheep polyclonal (RRID:AB\_10718118).

From Thermo Fisher Scientific (Waltham, MA): CD103 PerCP cy5.5 clone 2e7 (RRID:AB\_1574957), INOS APC780 clone CXNFT (RRID:AB\_2716962), Fixable blue, LIVE/DEAD, TNFa AF488 clone MP6-XT22 (RRID:AB\_469936), Ki67 Pecy7 clone SolA15 (RRID:AB\_11220070) and FoxP3 APC clone FJK-16S (RRID:AB\_469457).

**IHC and IF assay on mouse tumors models**

Anti-mouse CD8, CD4, and FOXP3 rat monoclonal antibodies used in IHC were obtained from eBioscience/Thermo Fisher Scientific (CD8 catalog #14-0808-82 [RRID:AB\_2572861]; CD4 catalog #14-9766-82 [RRID:AB\_2573008]; FoxP3 catalog #14-5773-82 [RRID:AB\_467576]). The rabbit anti-rat secondary antibody used for primary Ab detection was from Thermo Fisher Scientific (catalog #31219). Sections of formalin-fixed paraffin-embedded tissue were cut at 4 µm and placed on StarFrost® microscope slides. IHC was performed using a Leica Bond™ autostainer protocol (Leica Biosystems, Heerbrugg, Switzerland). Epitope retrieval solution 1 (Leica, catalog #AR9961) was used for antigen retrieval, and CD8, CD4, and FoxP3 mAbs were used at 0.1 µg/mL, 0.1 µg/mL, and 0.8 µg/mL, respectively, for primary antigen detection. Secondary antibody was applied at 5 µg/mL and slides were developed and counterstained with the Leica Bond Polymer Refine Detection kit (Leica, catalog #DS9800).

Anti-mouse CD163 rabbit monoclonal antibody (Abcam, catalog #ab182422; RRID:AB\_2753196) was applied at 0.674 µg/mL, followed by DISCOVERY® UltraMap anti-Rabbit Alkaline Phosphatase (Roche, catalog #760-4314) secondary Ab, and developed with DISCOVERY® Yellow Kit (Roche, catalog #760-239). Slides were counterstained on the Leica Bond™ autostainer with Hematoxylin II (Roche, catalog #790-2208) and Bluing Reagent (Roche, catalog #760-2037). All slides were scanned in an Aperio scanner (Leica) and digitally analyzed with Halo™ software by an experienced pathologist.

All samples for immunofluorescence assays were formalin-fixed and paraffin-embedded (FFPE) into blocks and sectioned at 4 µm with consecutive sections used for staining. All staining steps, including antigen retrieval were run on a Ventana Discovery Ultra. The following primary antibodies were first validated chromogenically and then titrated for dual staining of pSTAT3/CD45, pSTAT3/CD163, and pSTAT3/human mitochondria marker: phospho-STAT3 (CST, catalog #9145, clone D3A7 [RRID:AB\_2491009]) CD163 (Abcam, catalog #ab182422, clone EPR19518 [RRID:AB\_2753196]), CD45 (CST, catalog #70257, clone D3F8Q [RRID:AB\_2799780]), and anti-human mitochondria (Millipore, catalog #MAB1273, clone 113-1 [RRID:AB\_94052]). Primary antibodies were incubated for 1 hour followed by 32-minute incubation with a peroxidase labeled anti-rabbit or anti-mouse secondary (Ventana OmniMap anti-rabbit, catalog #760-4311, OmniMap anti-mouse, catalog #760-4310). Primary antibodies were sequentially stained, with a CC2 (pH 6.0) denaturation and Discovery Inhibitor (Ventana, catalog #760-4840) Peroxidase quench step in between. The following substrates were used for fluorescence detection: FAM (Ventana, catalog #760-243) for pSTAT3 and Red 610 (Ventana, catalog #760-245) for detecting CD45, CD163, and human mitochondria. The nuclear counterstain used was QD DAPI (Ventana, catalog #760-4196). CT26 samples were scanned with an Aperio Versa (Leica). PDX samples were scanned using a Zeiss Axioscan Z.1. Image analysis was performed on the HALO platform (Indica Labs).

**Single cell sequencing and data analysis**

*scRNAseq.* 3’ gene expression was profiled at the single cell level using the 10X Genomics Single Cell 3’v2 Reagent Kit (10X Genomics, Pleasanton, CA). CD45+ tumor-infiltrating immune cells were isolated using an EasySep™ Release Mouse PE Positive Selection Kit (catalog #17656; Stemcell Technologies, Vancouver, BC) after binding rat PE anti-mouse CD45 antibody (catalog #103106; Biolegend, San Diego, CA [RRID:AB\_312971]) to dissociated cells. Isolated cells were washed with PBS and diluted to 1,000 cells/µL in 1X PBS with 0.04% BSA. 20,000 cells were loaded per reaction and Gel Bead-in-Emulsions (GEMs) were generated with the 10X Genomics Chromium Controller, followed by reverse transcription, GEM-RT cleanup, and cDNA amplification per 10X protocol. 35 µL of cDNA was used as input into library construction. Samples underwent fragmentation, end repair and A-tailing, followed by adapter ligation, sample index PCR, and post-library QC per 10X protocol. Libraries were quantified on the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) using the KAPA Library Quantification Kit for Illumina Platforms with ROX Low qPCR Mastermix (KAPA Biosystems, Wilmington, MA, USA). Libraries were diluted to 1.5 nM and pooled in equal volumes with 6 libraries per pool. The pool was spiked with 1% PhiX, diluted to 300 pM, and sequenced on the Novaseq 6000 System with S2 flow cell (Illumina, San Diego, CA) with the following parameters: Read 1: 26 cycles; i7 Index: 8 cycles; Read 2: 98 cycles.

*scRNAseq data mapping, quantification and quality control.* Sequencing data was mapped and quantified using Cell Ranger software (v2.1.0) with default parameters. Downstream analysis was performed using the *Seurat* package (v2.3.4; RRID:SCR\_007322) (1) and plotted using ggplot2 (2) (RRID:SCR\_014601). Raw count matrices per sample were uploaded to R software (CreateSeuratObject, Seurat). A single Seurat object was created (MergeSeurat, Seurat), and counts were log-normalized with a scale factor of 10,000. Cells with more than 10% of mitochondrial reads and fewer than 300 genes were excluded. Due to the presence of cell types with very disparate median numbers of genes per cell, this permissive threshold of number of genes was used in order to avoid exclusion of any meaningful cell population. The number of unique molecular identifiers (UMIs) and percentage of mitochondrial reads were regressed out, and the resulting residuals were scaled (ScaleData, Seurat). Highly variable genes (HVGs) were identified based on the expression-corrected dispersion values (FindVariableGenes, Seurat; x.low.cutoff = 0.0125, x.high.cutoff = 2.5, y.cutoff = 1.05).

*scRNAseq dimensionality reduction, clustering and differential expression.* Scaled expression values for the HVGs were used as input for the Principal Component Analysis (RunPCA, Seurat). 30 PCs were then used for Uniform Manifold Approximation and Projection calculation (UMAP; RunUMAP, Seurat). The same number of PCs was used for cell clustering (FindClusters, Seurat), which was performed for a range of resolutions. Per resolution, differential expression genes were identified for each cluster vs all other cells (1 vs All), and for all pairwise comparisons between clusters (1 vs 1) (FindMarkers, Seurat; Wilcoxon test).

*Cell type filtering and subsetting.* A summary of the quality control and filtering steps is shown in Supplementary Figure 5A. Although CD45 enrichment was performed prior to scRNAseq, inspection of *Ptprc* (CD45) mean expression level and percentage of expressing cells revealed multiple CD45neg cell clusters. While some of these clusters corresponded to low quality cells, most CD45neg clusters exhibited high levels of *Tm4sf1* and multiple collagens, which are respectively characteristic of tumor and tumor-associated cell types. Cells in these clusters were excluded, and cells in the CD45+ clusters were reanalyzed as described above. Based on known markers (3), we chose the clustering resolution that allowed cells to be divided into: B cells (*Cd19*); T and NK cells (*Cd3e* and *Ncr1*, respectively); neutrophils (*Csf3r*); macrophages, monocytes and DCs (*Csf1r*); pDCs (*Siglech*); and basophils (*Mcpt8*). Differentially expressed genes can be found in Supplementary Table S4. For downstream analysis, cell types were grouped into “Lymphocytes” (B, T and NK cells) or “Mono.Macro.DC” (Macrophages, Monocytes, DCs and pDCs), and the same Seurat-based analysis was repeated. Differentially expressed genes among “Lymphocytes” or “Mono.Macro.DCs” for the resolutions chosen are listed in Supplementary Tables S5-S6. For “Mono.Macro.DCs”, which exhibit higher median number of genes, cells with fewer than 800 genes detected were excluded. Upon cell annotation within each group, small clusters of lower quality cells (low median number of genes and/or high mitochondrial content) or contaminant cells were further excluded from the analysis. To identify high-level populations, such as T and B cells, expression levels and fraction of expressing cells for known marker genes per cluster were inspected. While DC subpopulations match DC1-3 identified by Zilionis et al (3), Mono.Macro subpopulations were simply numbered from most to least abundant.

*Hierarchical clustering of subpopulations.* To assess the relationship between cell subpopulations, the intersection of the top 500 marker genes for all subpopulations (“1 vs All” comparisons; adjusted p-value < 0.01 and log fold-change > 0.3) was considered. The mean expression level of each gene per subpopulation was used as input for hierarchical clustering using ward.D2 clustering on Euclidean distances (pheatmap package; RRID:SCR\_016418) (4). To classify Mono.Macro subpopulations as M1/M2-like, gene signatures for these phenotypes were used. Specifically, M1 and M2-like macrophage clusters were grouped by hierarchical clustering using a gene list identified from *in vivo* stimulation of peritoneal macrophages to polarize to an M1 (LPS) or M2-like (no LPS) phenotype (5).

**Myeloid-derived suppressor cell (MDSC) cell suppression assay**

Gr-1+ cells were purified from CT26 tumors using anti-mouse Ly-6G and Ly-6C+ selection particles (Becton Dickinson, Franklin Lakes, NJ) and pooled by treatment group. CD3+ cells were purified using a CD3 negative selection kit (StemCell, Vancouver, Canada) from naïve mouse spleens. CD3+ cells were then labeled with 5 µM CFSE proliferation dye (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer’s protocol for suspension cells. CFSE-labeled T cells were co-cultured with Gr-1+ cells in several ratios (4:1, 2:1, 1:1, 1:2, and 1:4 T-cells to Gr-1 cells) for 96 hours to determine the suppressive capacity of the Gr-1+ cells. T cells were stimulated to proliferate using 10 ng/mL recombinant mouse IL2 (Peprotech, Rocky Hill, NJ), 10 µg/mL plate bound anti-mouse CD3 (clone 17A2; Biolegend, San Diego, CA [RRID:AB\_312658]), and suspended 10 µg/mL anti-mouse CD28 (clone 37.51; BioLegend, San Diego, CA [RRID:AB\_312866]). After the 96 hour incubation, cells were isolated from culture and bound to APC conjugated anti-CD3 (clone: 17A2, eBioscience, San Diego, CA [RRID:AB\_10597589]), BUV395 labeled anti-CD11b (clone: M1/70, BD Biosciences, Franklin Lakes, NJ ), BV785 conjugated anti-Ly6C (clone: HK1.4, eBioscience, San Diego, CA [RRID:AB\_2565852]), and PE conjugated anti-Ly6g (clone 1A8; eBioscience, San Diego, CA [RRID:AB\_2572720]). Cells were analyzed on a BD Symphony using BD FACSDiva software (BD, Franklin Lakes, NJ; RRID:SCR\_001456). The proliferation of T cells was measured by quantification of CFSE dilution by CD3+ cells analyzed on FlowJo™ flow cytometry software (FlowJo, Ashland, OR; RRID:SCR\_008520).

**Supplementary References**

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