SUPPLEMENTARY MATERIALS

Materials and Methods

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Fig. S2. Loss of sensitivity of primary cells to ASO over time when plated in culture.

Fig. S3. Activity of cEt (Gen 2.5) STAT3 ASO (AZD9150) and Gen 2.0 STAT3 ASO in tumor cells *in vitro* determined by immunoblot.

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Fig. S6. A decrease in the expression of STAT3-target genes after downregulation of STAT3 by STAT3 ASOs in ALCL, KARPAS299 cells.

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Fig. S8. Effect of STAT3 inhibition on cell proliferation in a variety of cell types *in vitro*.

Fig. S9. IHCs of ASOs and STAT3 in A431 xenograft tumors.

Fig. S10. Effect of AZD9150 on the growth of A431 xenograft tumors.

Materials and Methods

Study design

The main objective of this study was to evaluate the in vitro and in vivo pharmacological activity of AZD9150, a human STAT3 ASO with Gen 2.5 cEt chemistry in human cancer models. First, the potency of AZD9150 over Gen 2.0 STAT3 ASOs and its selectivity against other STAT proteins were assessed in a variety of human cancer cell lines *in vitro* by qRT-PCR, immunoblot, cell proliferation, and apoptosis assays after free uptake of the drug. These investigations were extended to multiple cell line- and patient-derived tumor xenograft models, where systemic administration of AZD9150 resulted in selective depletion of STAT3 mRNA and protein in tumors. Antitumor activity of AZD9150 was assessed by measuring tumor volume and changes in STAT3 downstream targets. Tumor-bearing animals were randomized before treatment on the basis of either tumor size (in s.c xenograft models) or the expression of biomarkers reflecting tumor burden (in dissemination models). Lastly, dose-escalation Phase I cancer studies were conducted with AZD9150 to assess the safety and the initial signs of clinical activity of the drug. Detailed information on inclusion criteria, dosing regimen, toxicity assessment, PET scan, PK, and IL-6 analyses is provided in the following sections.

Clinical study design

Inclusion criteria included age 18 years or older; tumors that were relapsed or refractory to at least one prior anticancer systemic therapy and for which no standard therapy exists; measurable disease

by Response Evaluation Criteria in Solid Tumors (RECIST) or International Working Group response criteria for malignant lymphoma; ECOG performance status <=2; and if of childbearing potential, willingness to use adequate contraception. Exclusion criteria included active or uncontrolled infection; absolute neutrophil count <1000/μL; platelet count <100,000/μL; hemoglobin <9 g/dL; total bilirubin > 2x upper limit of normal (ULN); AST and ALT > 3x ULN; creatinine > 2 mg/dL; New York Heart Association Class II or greater congestive heart failure; history of myocardial infarction within 6 months of screening; brain metastases; therapy within 4 weeks of screening; prior STAT3 inhibitor treatment; and pregnancy.

Isis Pharmaceuticals supplied AZD9150. All patients signed a written informed consent meeting requirements of institutional review boards (MD Anderson Cancer Center, Memorial Sloan-Kettering Cancer Center, and Mary Crowely Cancer Research Center). A standard 3 + 3 dose-escalation design was used. AZD9150 was administered intravenously as a loading dose on days 1, 3, and 5 of the first week followed by weekly dosing given in 3-week cycles. Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 4.0. Dose-limiting toxicity (DLT) was defined as any non-hematological toxicity greater than or equal to Grade 3 that was not adequately controlled by supportive care measures, Grade 4 neutropenia lasting more than 7 consecutive days, or Grade 4 thrombocytopenia or thrombocytopenia requiring platelet transfusion. Exceptions were Grade 3 fever, chills, fatigue lasting < 7 days, aPTT prolongation without hemorrhage, asymptomatic elevation of ALT, lab abnormalities that in the opinion of the Investigator has no clinical consequence, and alopecia. The DLT window

encompassed the first 28 days of treatment. The MTD was defined as the dose level in which fewer than 1 of 6 patients experienced a DLT. Baseline evaluations were conducted within 4 weeks of initial dosing. Physical exams were conducted every week and hematologic/biochemical laboratories were performed before every dose. FDG-PET scans were required within 4 weeks before therapy. RECIST or IWGC evaluations were performed at week 7 and then every 6 weeks thereafter. Patients continued on treatment until disease progression, unacceptable adverse event, intercurrent illness preventing further administration, or patient withdrawal.

Plasma drug concentrations were assessed on day 1 and optionally on day 15 at 0, 1.5, 3, 3.5, 4, 6, 8, and 24 hours after the start of the infusion. Additional pre-dose concentrations were assessed on days 3 and 5 of the loading week and then weekly thereafter. The plasma concentration of AZD9150 was determined by a validated high-performance chromatography-UV method as described previously (*59*).

Serum was collected on day 1 and day 15 and then every three weeks from day 29. IL-6 was measured by a validated ELISA assay by AushonBioSystems, Inc.

Cell culture and reagents

All the cell lines were purchased from American Type Culture Collection (ATCC) with the following exceptions: SUP-M2 and KARPAS299 lines were from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), and PC-9 cells were from IBL. All cells were tested for mycoplasma before use. Mouse primary hepatocytes were isolated and cultured as previously

described (*60*). The cell lines were cultured in the recommended medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin in a 5% CO_2 humidified incubator at 37 °C. AZD1480 and crizotinib were obtained from Selleckchem.

Antisense oligonucleotides (ASOs)

Gen 2.0 ASOs with 2'-methoxyethyl (MOE) or Gen 2.5 ASOs with cEt chemistry were synthesized as described previously (*14, 61*). STAT3 ASOs were designed to be either cross-species or species-specific, and the lead compounds were identified by screening ~500 ASOs against the gene. The sequences of Gen 2.5 STAT3 ASOs used for the study were AZD9150, 5'-CTATTTGGATGTCAGC-3', and ASO-2, 5'-GAAATTCATTCTTCCA-3', with underlined letters indicating cEt modified bases. Gen 2.0 human STAT3 ASO (5'-CAGCAGATCAAGTCCAGGGA-3') and two Gen 2.0 human STAT3 ASOs with identical sequences to that of AZD9150 (5'-CTATTTGGATGTCAGC-3' and 5'-TTCTATTTGGATGTCAGCAA-3') were modified with MOE as indicated by underlined letters. Scrambled ASOs were included in each study as negative controls. In experiments to measure cell proliferation, both scrambled (5'-GGCTACTACGCCGTCA-3') and Eg5 (5'-CCGAGCTCTCTTATCA-3') ASOs with Gen 2.5 modified bases were included as negative and positive controls, respectively. To demonstrate the loss of sensitivity of primary cells to ASO *in vitro* over time, mouse MALAT1 ASO (5'-CCAGGCTGGTTATGACTCAG-3') with MOE-modified bases underlined was tested in mouse primary hepatocytes. siRNAs for STAT3 were purchased from Dharmacon (J-003544-07 and J-003544-08).

Delivery of ASOs *via* free uptake for *in vitro* experiments

Cells were seeded on collagen I-coated 96-well or 6-well plates (BD Biosciences) 16 hours before treatment. ASO prepared in PBS at 1 mmol/L was subjected to serial dilution with complete growth medium (10% FBS) to achieve the indicated final concentrations and added to the cells directly without using any transfection regents. Cells were harvested 1-3 days (for RNA) or 1-5 days (for protein) after treatment depending on the end points of each experiment. In an experiment where the delivery of ASOs was compared to that of siRNAs, RNAiMAX (Life Technologies) was used to transfect the cells. To deliver STAT3 siRNAs, KARPAS299 and SUP-M2 cells ($1x10^6$ cells) were electroporated using BTX's ECM830 (at 220V for 8.5 ms, 3 pulses). The IC_{50} values were calculated using the GraphPad Prism software.

qRT-PCR and immunoblot analyses

Total RNA from either cell culture or tumor samples was isolated with the Qiagen RNeasy 96 plate kit or RNeasy Mini kit following the manufacturer's protocol. qRT-PCR was performed by ABI Prism 7700 Sequence Detector or ABI StepOne Plus. The abundance of each target mRNA was measured and normalized using species-specific probe/primer sets purchased from ABI or shown in table S5. Protein lysates from either cell culture or tissues were prepared in radioimmunoprecipitation assay buffer (Sigma Chemicals Co.) or Phosphosafe Extraction Reagent (Novagen) containing both protease and phosphatase inhibitor cocktails. The samples were then separated on bis-Tris 4-12% gradient SDS-PAGE using MOPS running buffer (Life

Technologies), transferred onto polyvinylidenedifluoride (PVDF) membranes (GE Healthcare) or nitrocellulose membranes (InVitrogen iBlot), and probed with primary antibodies against STAT3 (Cell Signaling Technology #9132, #9139, #4904; Abcam #32500; BD Biosciences #610189), phospho-STAT3 (Tyr705; Cell Signaling Technology #9145), STAT1 (Cell Signaling Technology #9172), STAT5 (Cell Signaling Technology #9363), MCL-1 (Cell Signaling Technology #4572), c-MYC (Santa Cruz Biotechnology #sc-40), VEGF (Santa Cruz Biotechnology #sc-152), cleaved PARP (Cell Signaling Technology #5625), β-Actin (Millipore #MAB1501), and GAPDH (Advanced Immunochemicals Inc. #6C5 or Cell Signaling Technology #2118) in 0.05% Tween20-Tris Buffered Saline (T-TBS) containing 5% of either skim milk or BSA at 4 °C overnight. Secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) were used at 1:3000 to detect primary antibodies, and immune-specific bands were visualized by the Enhanced Chemiluminescence Plus detection kit (GE Healthcare). The intensity of STAT3 signal was quantified using the ImageJ program. In some cases, LI-COR #926-32210 or #926-32211 was used as the secondary antibody, and the images were captured and analyzed with Licor imager and software (LI-COR Biosciences).

Cell proliferation and apoptosis assays

Human cancer cells were plated in 96-well plates (1-10,000 cells/well) and incubated overnight. ASOs, siRNAs, or AZD1480 were added at various concentrations. Four to six days later, AlamarBlue (Invitrogen) or CellTiter AQueous one solution (Promega) was added, and the cells were incubated at 37 °C for 30 min to 2 hours. Cell proliferation was assessed by measuring

fluorescence or optical density at 490 nM. Apoptosis induced by STAT3 ASOs was assessed by FACS analysis using FITC-labeled active caspase-3 apoptosis kit (BD biosciences #550480).

Immunohistochemistry of STAT3 and ASO

Tumors were fixed in 10% neutral buffered formalin (Fisher) for 24 to 48 hours, transferred to 70% ethanol, embedded in paraffin, and sectioned at 4-µm thickness. IHC was performed on the Ventana Discovery XT Autostainer with the antibodies for total STAT3 (79D7, Cell Signaling Technology #4904) at 1:100 dilution, STAT5 (Abcam #194898) at 1:50 dilution, and STAT1 (Cell Signaling Technology #9175) at 1:200 dilution followed by detection with a donkey anti-rabbit biotinylated FAB2 secondary antibody (Fitzgerald International Industries, #43R-ID007bt) at 1:100 dilution. Secondary antibodies were detected with a Ventana DABMap kit (#760-124). Slides were then counterstained with hematoxylin (Ventana #760-2021), dehydrated, cleared, mounted, and examined using a light microscope. Intensity of STAT protein signal on IHC was calculated using Aperio's image analysis algorithms. IHC for ASO was performed as described (*36*).

Determination of tissue concentrations of ASOs

The methods of determining tissue concentration of oligonucleotides are modifications of previously reported methods (*59*). Briefly, tissues were homogenized and then extracted using a phenol-chloroform liquid-liquid extraction method. After this, solid-phase extraction of the supernatant was performed using a phenyl-bonded SPE column (Biotage). Extracted samples were

analyzed by ultra-high performance liquid chromatography (uHPLC) using an Agilent 1290 Infinity (Agilent Technologies). Calibration curves were established by plotting the peak area ratios (drug/internal standard) versus known concentrations of the oligonucleotides. Quantification of samples was performed using the calibration curves and the peak area ratios.

Xenograft studies

Mice were housed under pathogen-free conditions in individual ventilated cages (IVC) at our Assessment and Accreditation of Laboratory Animal Care (AALAC)-accredited facility. All animal manipulations were conducted in a biosafety cabinet maintained under positive pressure. All animal studies were conducted in accordance with the guidelines established by the internal Institutional Animal Care and Use Committee (IACUC). The original data for individual animals from the studies where significant antitumor effects of ASOs were observed are shown in table S6.

In the A431 tumor model, 6-to-8 week-old female Balb/c nude mice were inoculated subcutaneously with A431 cells in the flank region (5×10^6 cells/animal). When the mean tumor size reached approximately 100 mm^3 (on day 9), the tumor-bearing animals were treated with STAT3 ASOs subcutaneously at either 25 to 50 mg/kg (for Gen 2.5 ASO) or 50 mg/kg (for Gen 2.0 ASO), 5 times per week for 3 weeks (pharmacodynamics experiment, Fig. 2). Dose-dependent studies in A431 tumor model were repeated three times. For the efficacy study (fig. S10), A431 tumor-bearing animals were treated with Gen 2.5 STAT3 ASO (AZD9150) at 25, 37.5, or 50 mg/kg along with a control ASO at 50 mg/kg, 5 times per week for 3 weeks.

For the primary explant studies, primary human tumor samples were grown in female Charles River CB17-SCID (NSCLC-LN PDX and colorectal cancer PDX), female Charles River SCID-beige (lymphoma PDX pharmacodynamic experiment, Fig. 4), or female Jackson Labs NSG (lymphoma PDX efficacy experiment, Fig. 5) mice by serial subcutaneous transplantation of tumor fragments. Mice bearing passage 5 to 7 tumors were treated subcutaneously with Gen 2.5 STAT3 ASOs at 50 mg/kg, 5 times per week for 3 weeks, beginning when tumor volumes were in the range of 50 – 150 mm^3 (colorectal cancer PDX and lymphoma PDX), or 250 – 1200 mm^3 (NSCLC-LN PDX).

For the SUP-M2 xenograft study, 4 to 6-week-old female NOD/SCID mice were implanted with human anaplastic large cell lymphoma (ALCL), SUP-M2 cells (5 x 10^6 cells/animal). When the mean tumor volume reached ~150 mm^3, the tumor bearing animals were treated with Gen 2.5 STAT3 ASO or control ASO at 50 mg/kg, 5 times per week for 5 weeks. For SUP-M2 dissemination model, the cells were intravenously injected into 5 week-old NSG mice (5 x 10^6 cells/animal). Twenty days later, animals were randomized on the basis of soluble CD30 (sCD30) concentration and treated with either control ASO or Gen 2.5 STAT3 ASO at 50 mg/kg, 5 times per week for 2 weeks. Tumors from various tissues and the blood were collected at the end of study to assess *STAT3* concentration and tumor burden. Soluble human CD30 concentrations measured by ELISA (eBioscience #BMS240) on day 20 and day 33 were used to randomize the animals before STAT3 ASO treatment and to assess the tumor burden, respectively. Soluble

human IL-2Rα concentrations were assessed by ELISA (R&D Systems #DR2A00) on day 33 as a downstream pharmacodynamic marker for STAT3 ASO.

For DLBCL PDX model, 10-week-old female NSG mice were implanted with tumor fragments subcutaneously in the flank (n=8 mice per group) and treated with Gen 2.5 STAT3 ASO at 50 mg/kg, 5 times per week when tumor sizes reached 175 mm^3.

For PC-9 tumor study, 9-week-old female 129S1/SvImJ mice were implanted with human NSCLC PC-9 cells (1 x 10^7 cells/animal) subcutaneously in the flank. Treatment with Gen 2.5 STAT3 ASO started at 25 mg/kg, 5 times per week when tumors reached a mean volume of 98 to 155 mm^3 (n=7 mice per group).

Statistical analysis

The two-tailed Student's t test was used to calculate *p* values for comparisons between treatment groups. *P* values of < 0.05 were considered to be statistically significant. Exact *P* values are shown in the text and figure legends.

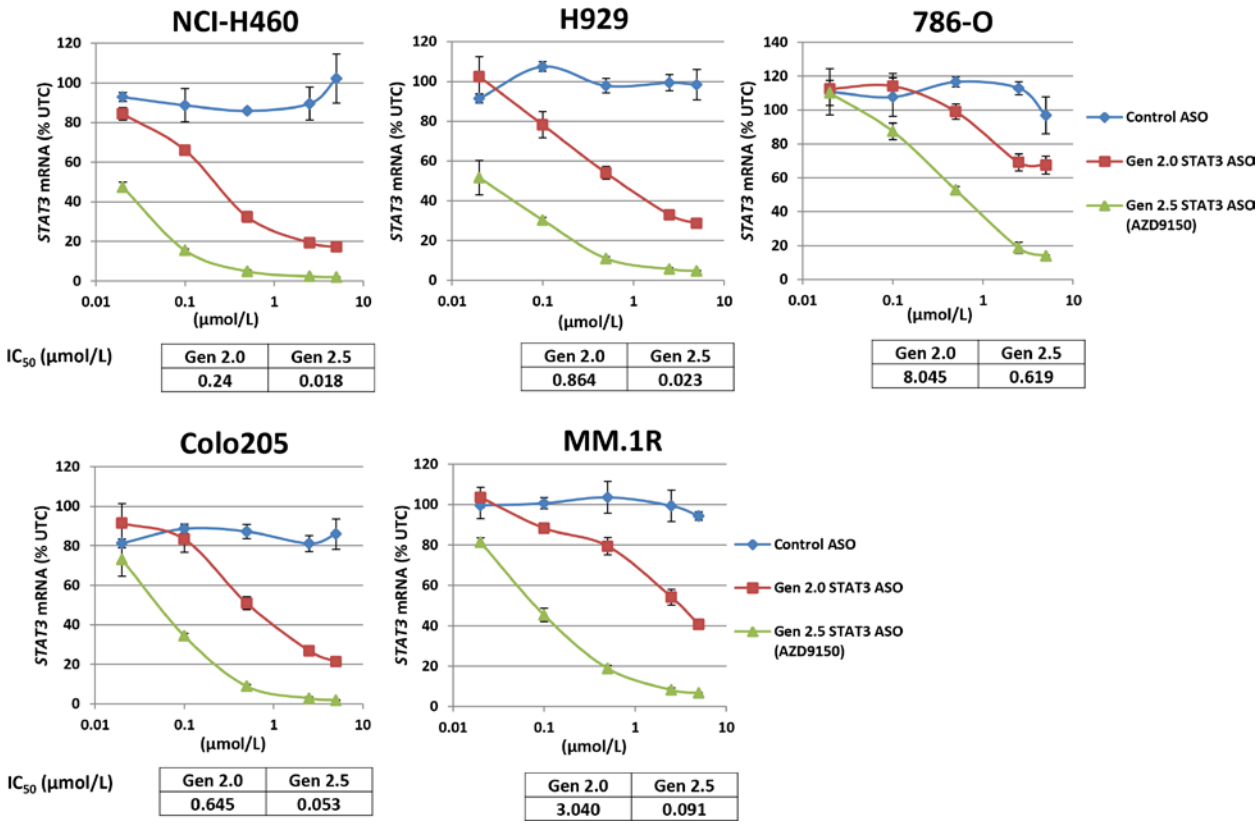


Fig. S1. Activity of cEt (Gen 2.5) STAT3 ASO (AZD9150) and Gen 2.0 STAT3 ASO in tumor cells *in vitro* determined by qRT-PCR. Gen 2.0 MOE or cEt STAT3 ASO along with control ASO were delivered to a variety of human cancer cells *via* free uptake in 96-well plates in triplicate for each test condition. RNA was isolated 24 hours later, and *STAT3* knockdown was determined by q-RT-PCR. IC_{50} values for Gen 2.0 and Gen 2.5 cEt STAT3 ASOs calculated using GraphPad Prism software are shown for each cell line. UTC: untreated cells. These experiments have been replicated at least 3 times. Graphs show the means ± SD of n =3.

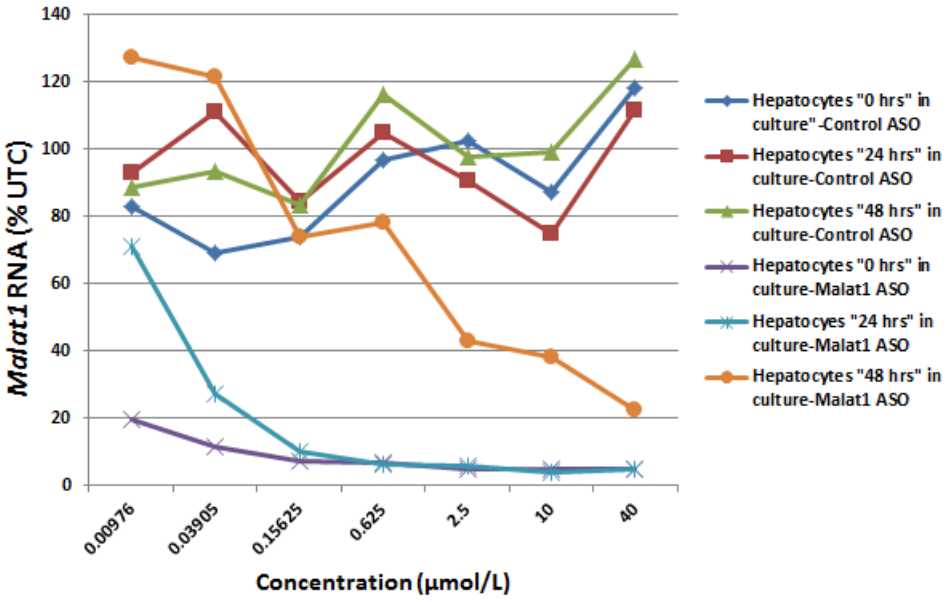


Fig. S2. Loss of sensitivity of primary cells to ASO over time when plated in culture. Freshly isolated mouse hepatocytes were plated on collagen-coated plates and then either, treated immediately (0 hrs) with a range of concentrations of test ASOs (Malat1 ASO or Control ASO) or incubated an additional 24 or 48 hrs in culture before treatment with test ASOs. The sensitivity of hepatocytes maintained in cell culture for different periods (0, 24 or 48 hrs) to the test ASOs was determined by their ability to decrease the level of *Malat1* RNA, measured by qRT-PCR. *Malat1* levels are represented as % of untreated cells (UTC).

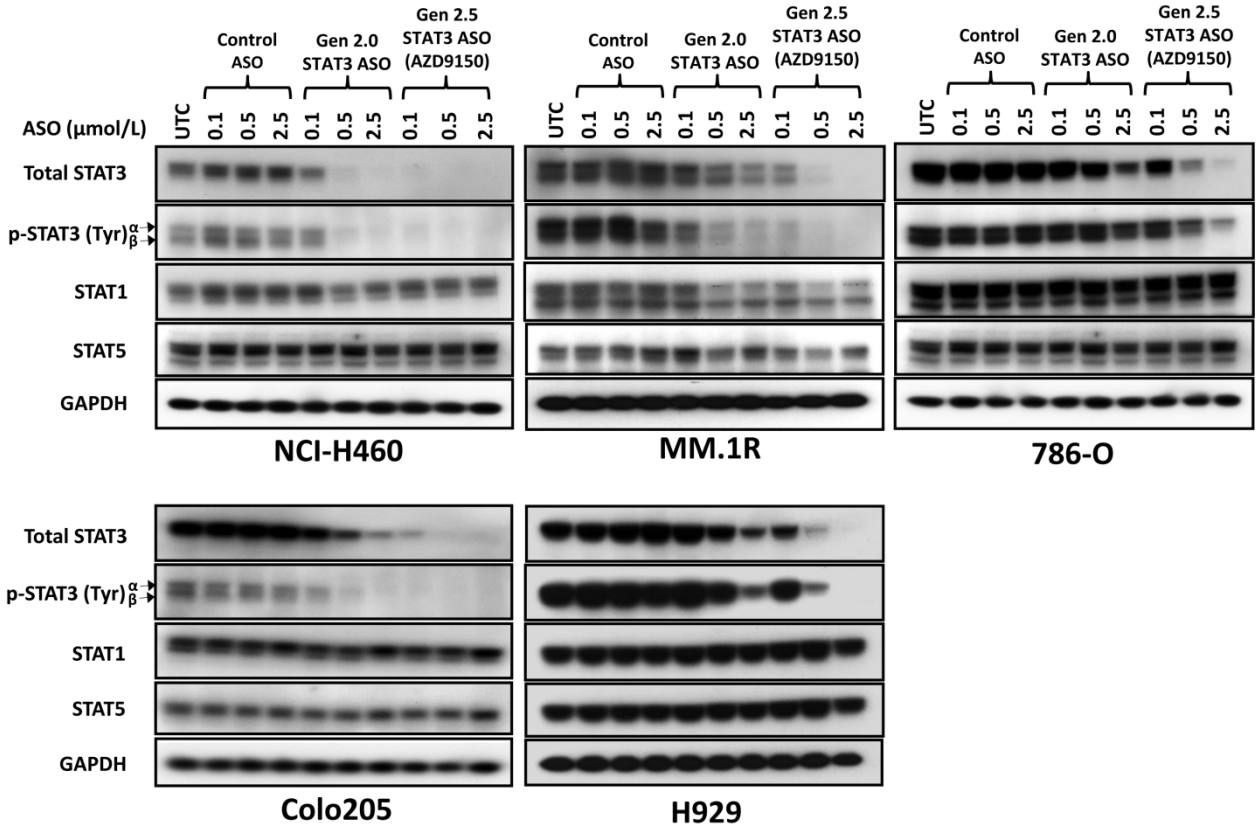


Fig. S3. Activity of cEt (Gen 2.5) STAT3 ASO (AZD9150) and Gen 2.0 STAT3 ASO in tumor cells *in vitro* determined by immunoblot. Total cell lysates were collected for immunoblot analysis 3 days after ASO treatment. cEt STAT3 ASO (AZD9150) reduced both total and phosphorylated forms of STAT3 protein to a near undetectable level, at much lower concentration than Gen 2.0 STAT3 ASO, and had little effect on the other STAT members, STAT1 and STAT5. UTC: untreated cells.

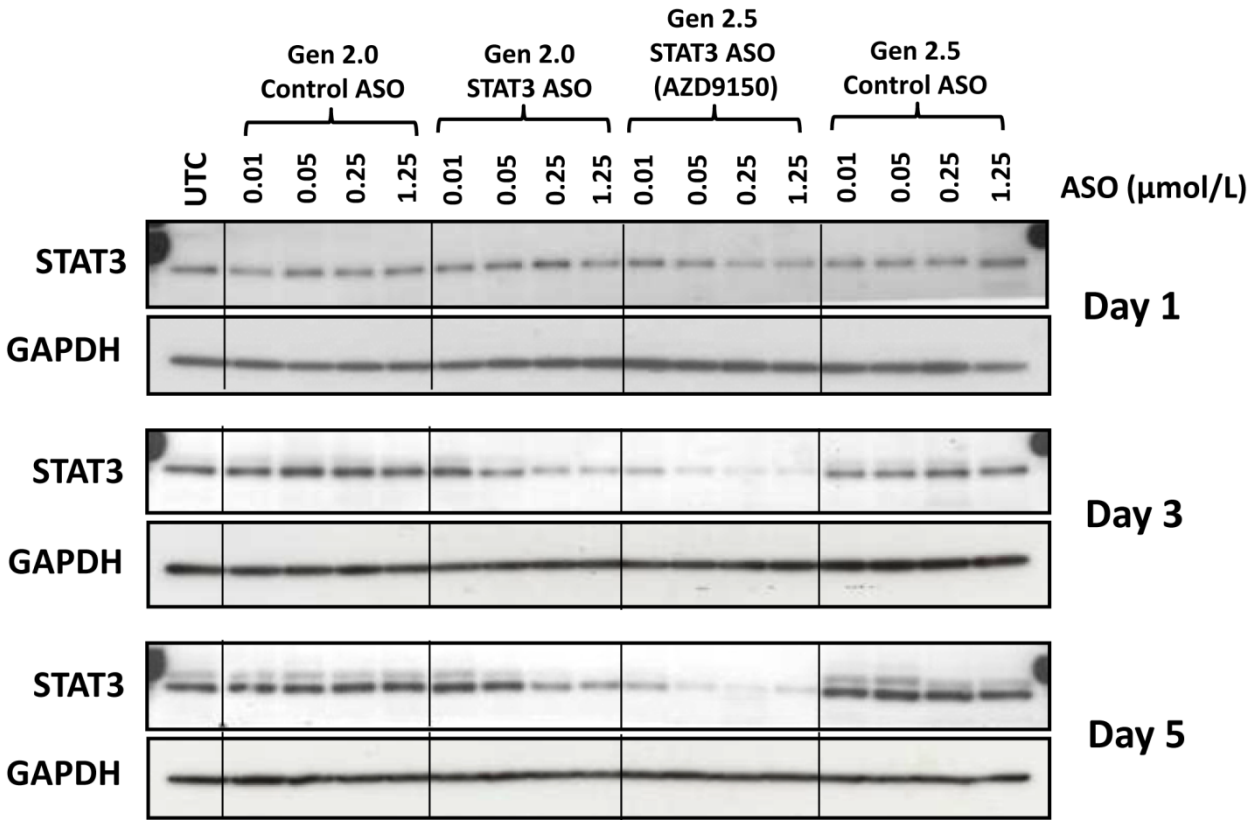


Fig. S4. Superior activity of cEt (Gen 2.5) STAT3 ASO (AZD9150) over Gen 2.0 STAT3 MOE ASO. Increasing concentration of STAT3 ASOs with two different chemistries along with control ASOs were delivered to A431 cells via free uptake, and the cells were harvested at different time points for STAT3 immunoblot analysis. The greater STAT3 downregulation by cEt STAT3 ASO compared to Gen 2.0 STAT3 ASO was demonstrated in both dose- and time-dependent fashions during the course of the experiment, whereas control ASOs had no effect on STAT3 concentration. UTC: untreated cells.

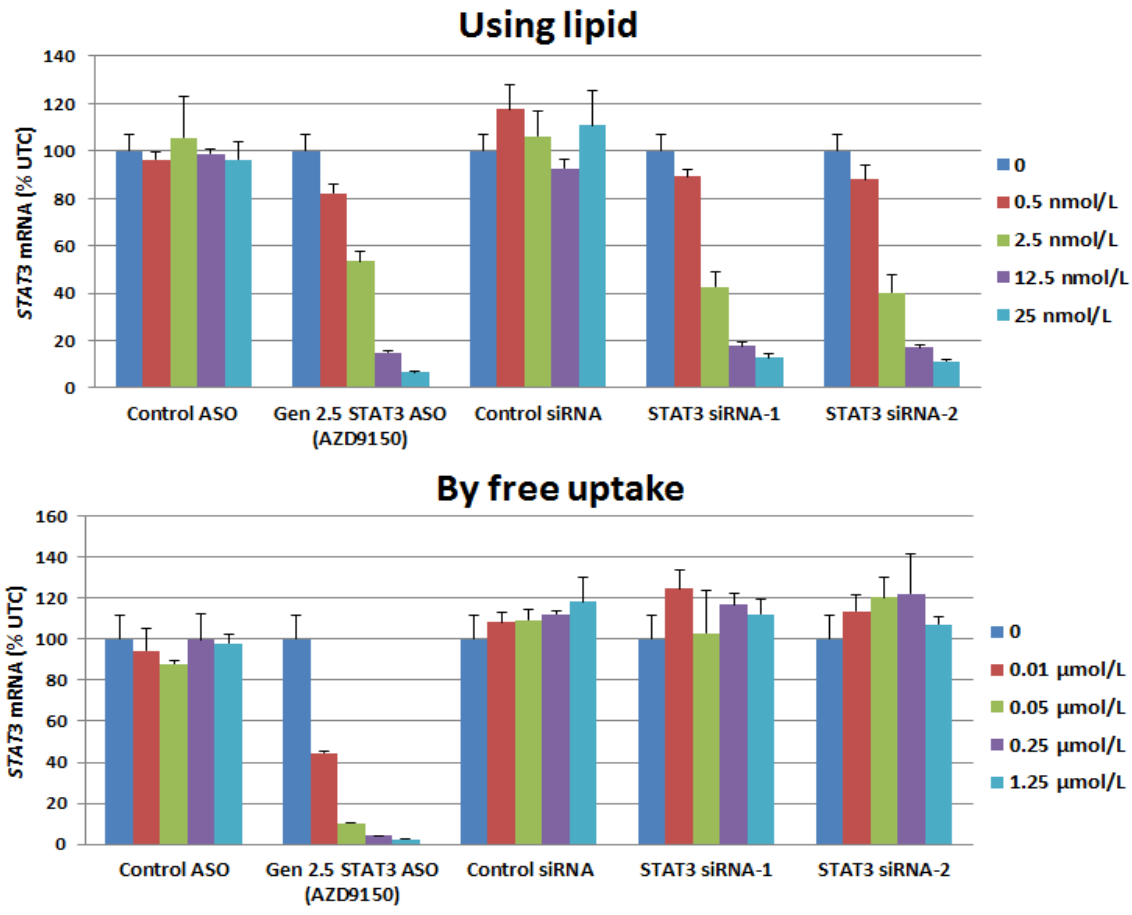


Fig. S5. Lack of activity of STAT3 siRNAs when delivered to tumor cells by free uptake. Increasing concentration of cEt ASO or double-stranded siRNAs for *STAT3* gene along with appropriate controls were delivered to A431 cells by either free uptake or using RNAiMAX transfection reagent. *STAT3* mRNA measured by qRT-PCR 24 hours later showed no target knockdown with STAT3 siRNAs delivered to the cells *via* free uptake, whereas cEt STAT3 ASO decreased *STAT3* in a dose-dependent manner. Triplicates were used for each ASO or siRNA

concentration and the experiments were performed twice. Graphs show the means ± SD of n =3. UTC: untreated cells.

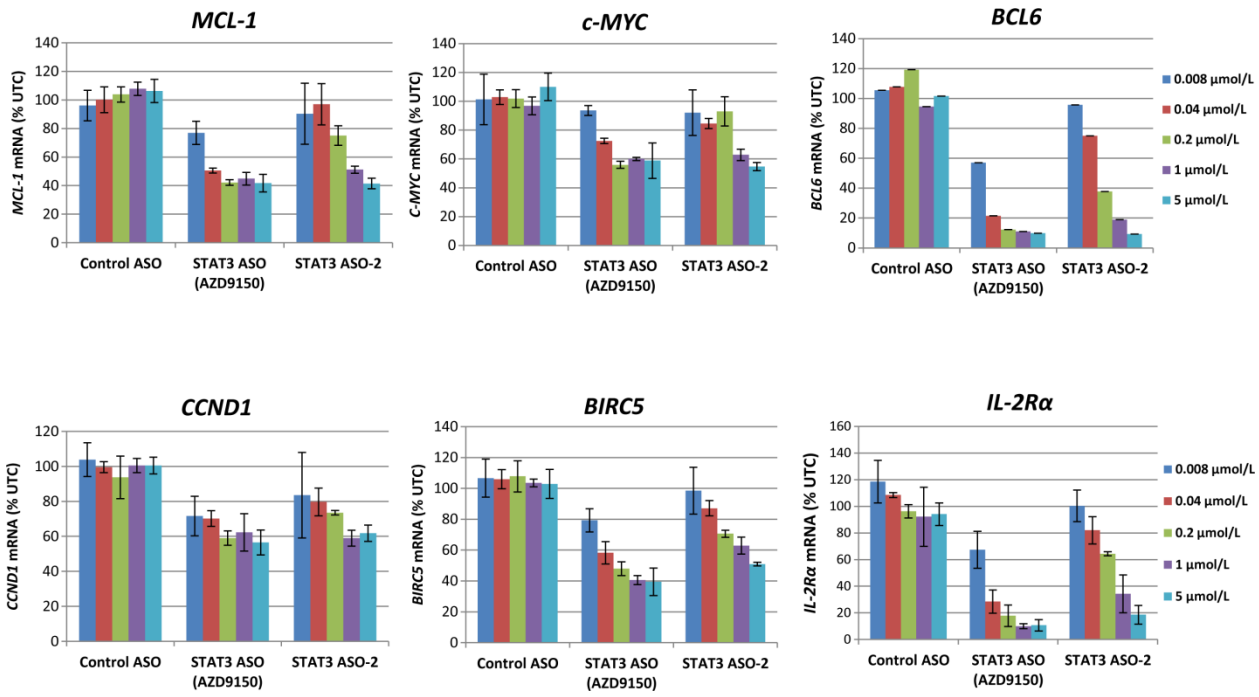
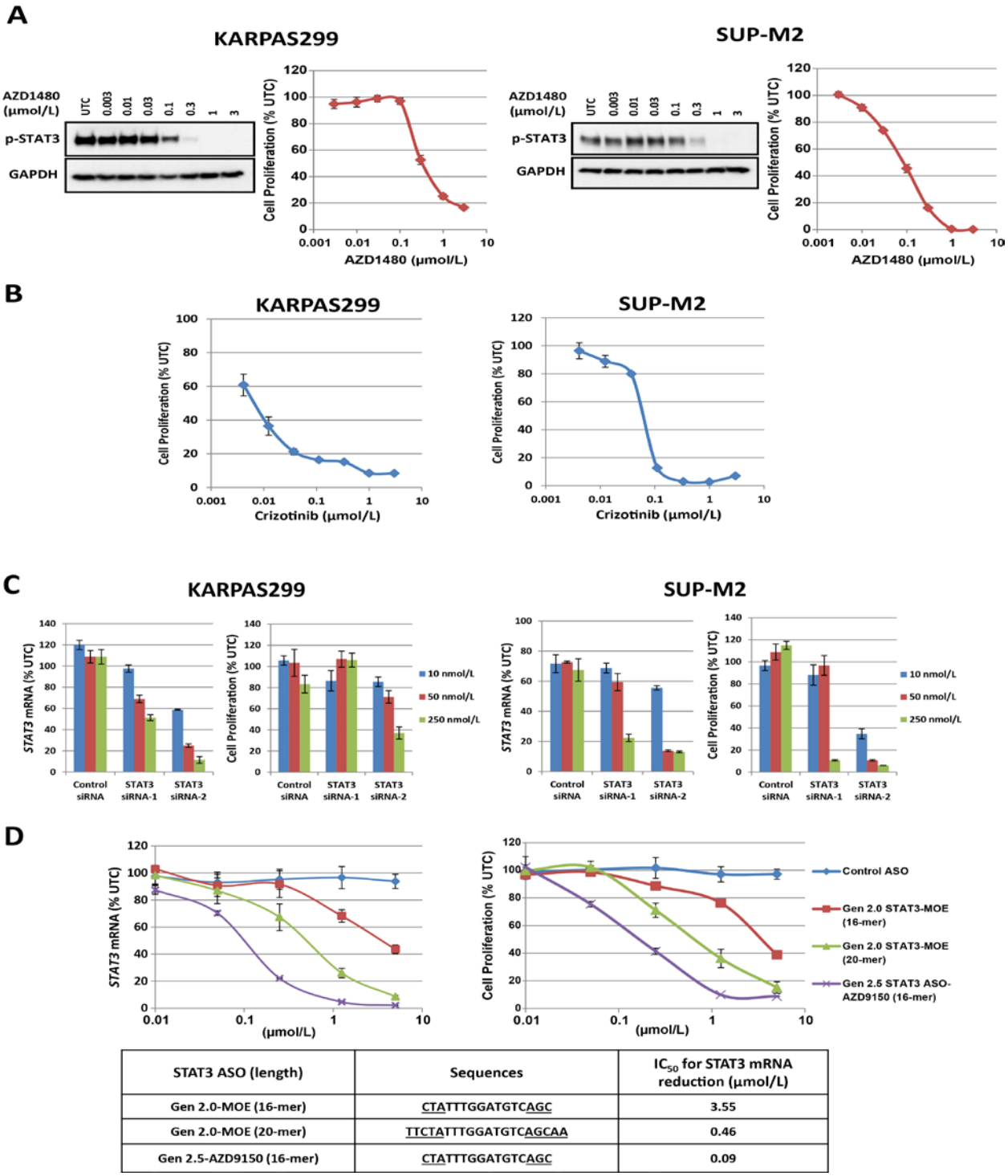
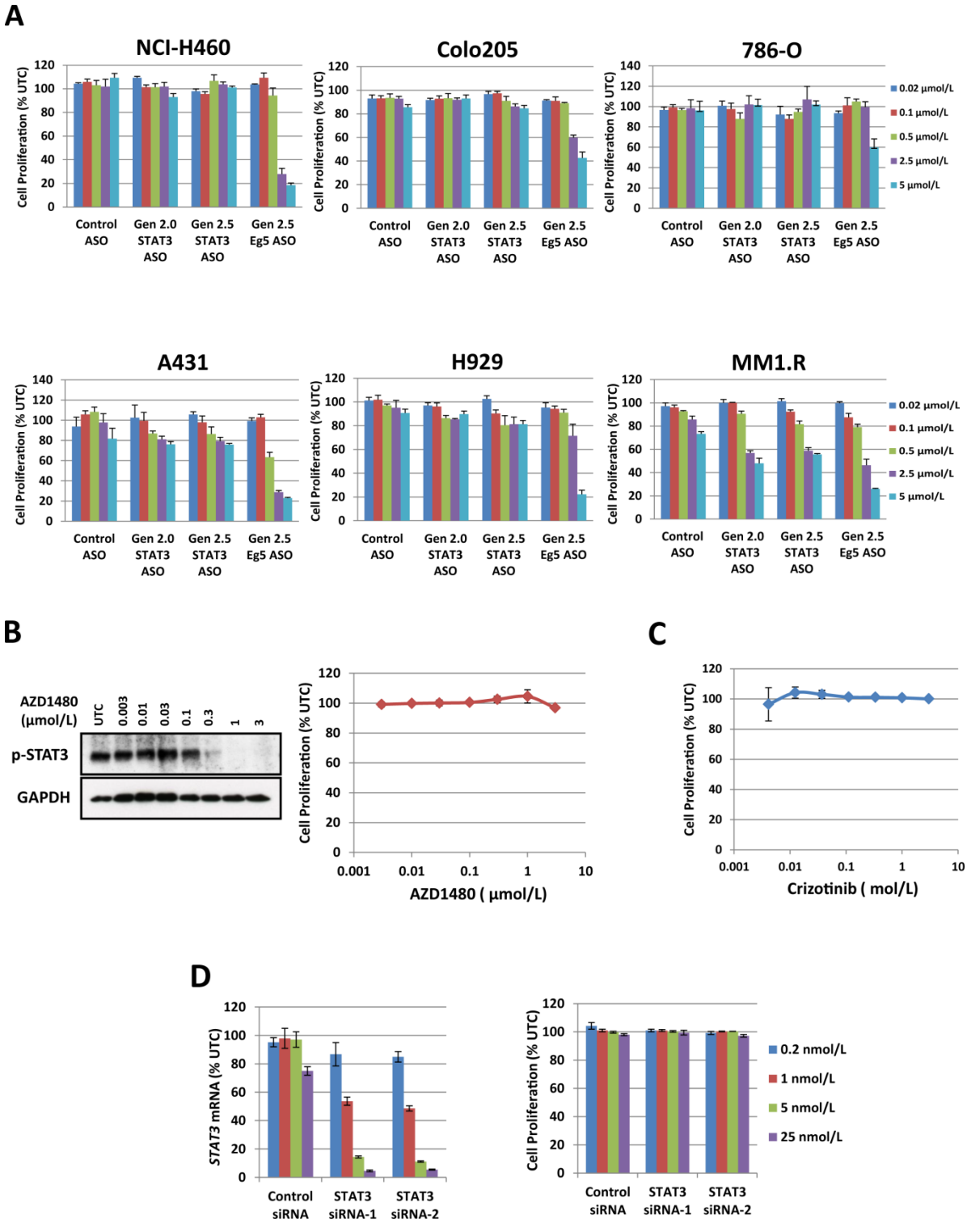


Fig. S6. A decrease in the expression of STAT3 target genes after downregulation of STAT3 by ASOs in KARPAS299 cells. Two cEt STAT3 ASOs along with a control ASO were delivered to KARPAS299 cells (ALCL) *via* free uptake. Expression of STAT3 target genes was measured by qRT-PCR on day 3. Graphs show the means ± SD of n = 3. UTC: untreated cells.



STAT3 ASO (length)	Sequences	IC_{50} for STAT3 mRNA reduction (μmol/L)
Gen 2.0-MOE (16-mer)	CTATTTGGATGTCAGC	3.55
Gen 2.0-MOE (20-mer)	TTCTATTTGGATGTCAGCAA	0.46
Gen 2.5-AZD9150 (16-mer)	CTATTTGGATGTCAGC	0.09

Fig. S7. Sensitivity of ALCL cell lines, KARPAS299 and SUP-M2, to STAT3 inhibition. (**A**) KARPAS299 and SUP-M2 cells were treated with increasing concentration of AZD1480 for either 1 hour for p-STAT3 immunoblot or 5 days for cell proliferation. (**B**) The ALCL cells were treated with increasing concentration of ALK inhibitor, crizotinib, for 3 days for the cell proliferation assay. (**C**) Two STAT3 siRNAs along with a control siRNA were delivered to the ALCL cells by electroporation. *STAT3* mRNA concentrations were measured 2 days later, and cell proliferation was assessed 5 days after treatment. (**D**) SUP-M2 cells were treated with STAT3 ASOs with different chemistries and lengths *via* free uptake. All STAT3 ASOs reduced *STAT3* mRNA expression in a dose-dependent manner, with AZD9150 being most potent, which resulted in a corresponding decrease in cell proliferation. The underlined sequences indicate either MOE- or cEt-modified bases for Gen 2.0 and Gen 2.5 chemistries, respectively. Graphs in A-D show the means ± SD of n =3. UTC: untreated cells.


A
NCI-H460
Colo205
786-O
A431
H929
MM1.R
Cell Proliferation (% UTC)
Control ASO
Gen 2.0 STAT3 ASO
Gen 2.5 STAT3 ASO
Gen 2.5 Eg5 ASO
0.02 µmol/L
0.1 µmol/L
0.5 µmol/L
2.5 µmol/L
5 µmol/L
B
AZD1480 (µmol/L)
UTC
0.003
0.01
0.03
0.1
0.3
1
3
p-STAT3
GAPDH
AZD1480 (µmol/L)
C
Crizotinib (mol/L)
D
STAT3 mRNA (% UTC)
Control siRNA
STAT3 siRNA-1
STAT3 siRNA-2
0.2 nmol/L
1 nmol/L
5 nmol/L
25 nmol/L

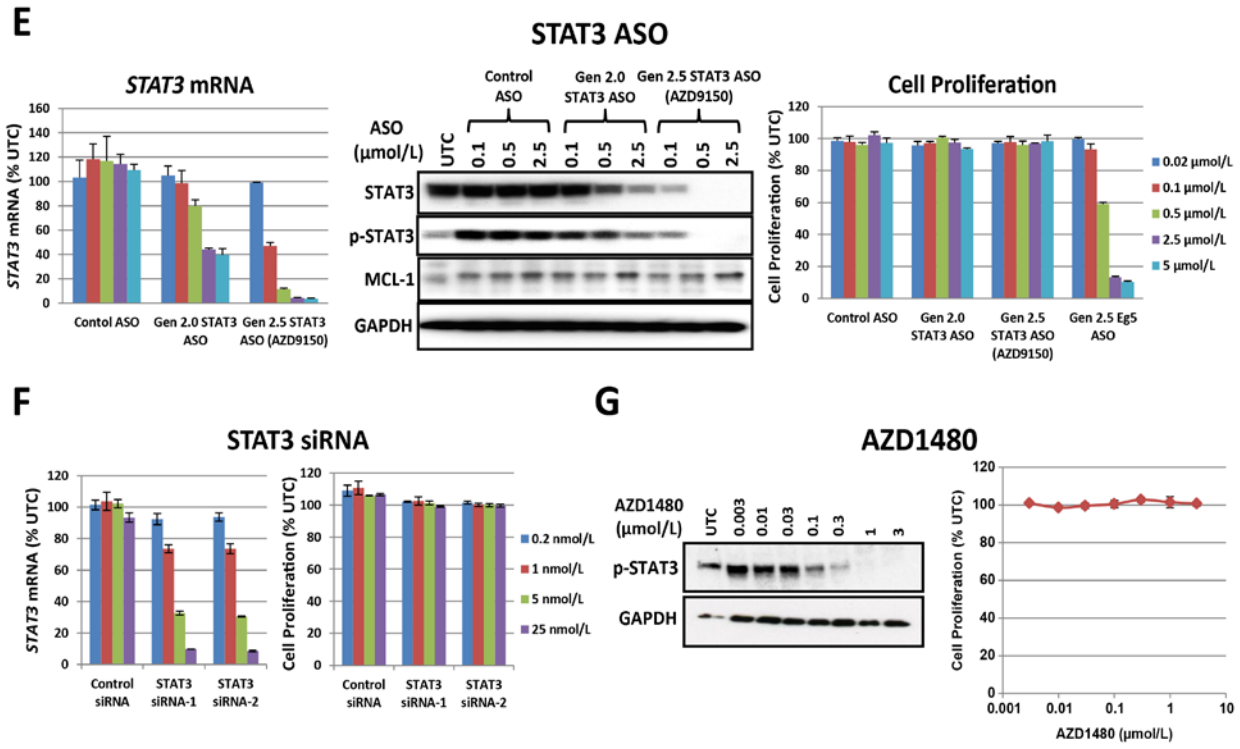


Fig. S8. Effect of STAT3 inhibition on cell proliferation in a variety of cell types *in vitro*. (**A**) Increasing concentrations of Gen 2.0 and cEt (Gen 2.5) STAT3 ASOs along with control ASOs were delivered to a variety of adherent (NCI-H460, Colo205, 786-O, and A431) or suspension (H929 and MM1.R) human cancer cells seeded in 96-well plates *via* free uptake. Cell proliferation was measured by MTS-based assay on day 5. Whereas the ASO for a mitotic target, Eg5, markedly inhibited cell proliferation in all the cell types tested as predicted, STAT3 ASOs had little effect on cell growth, irrespective of their chemistry, despite inducing strong STAT3 knockdown (as shown in Fig. S1). (**B**) NCI-H460 cells were treated with increasing concentration of AZD1480 for either 1 hour for p-STAT3 immunoblot or 5 days for cell proliferation assay.

Despite strong reduction in p-STAT3 (*left*), cell proliferation was not affected by the inhibitor (*right*). (**C**) NCI-H460 cells were treated with increasing concentration of ALK inhibitor, crizotinib, and cell proliferation was measured 3 days later. (**D**) NCI-H460 cells were treated with two STAT3 siRNAs along with a control siRNA. A marked reduction in *STAT3* mRNA measured on day 2 (*left*) did not decrease cell proliferation on day 5 (*right*). (**E**) PC-9 cells were treated with Gen 2.0 or cEt STAT3 ASO (AZD9150) along with a control ASO *via* free uptake. Reductions in *STAT3* mRNA and protein were measured by qRT-PCR (on day 1) and immunoblot analysis (on day 3), respectively. Despite strong reduction in STAT3 by STAT3 ASOs, cell proliferation was minimally affected on day 5, whereas the Eg5 ASO, used as a positive control in the experiment, inhibited cell proliferation as predicted. (**F**) PC-9 cells were treated with increasing concentration of STAT3 siRNAs. A marked reduction in *STAT3* mRNA determined on day 2 (*left*) did not result in any significant change in cell proliferation when measured on day 5 (*right*). (**G**) Despite strong reduction in p-STAT3 (*left*), proliferation of PC-9 cells was not affected by AZD1480 (*right*). Triplicates were used for each ASO concentration, and the experiments were performed twice. Graphs in A-G show the means ± SD of n =3. UTC: untreated cells.

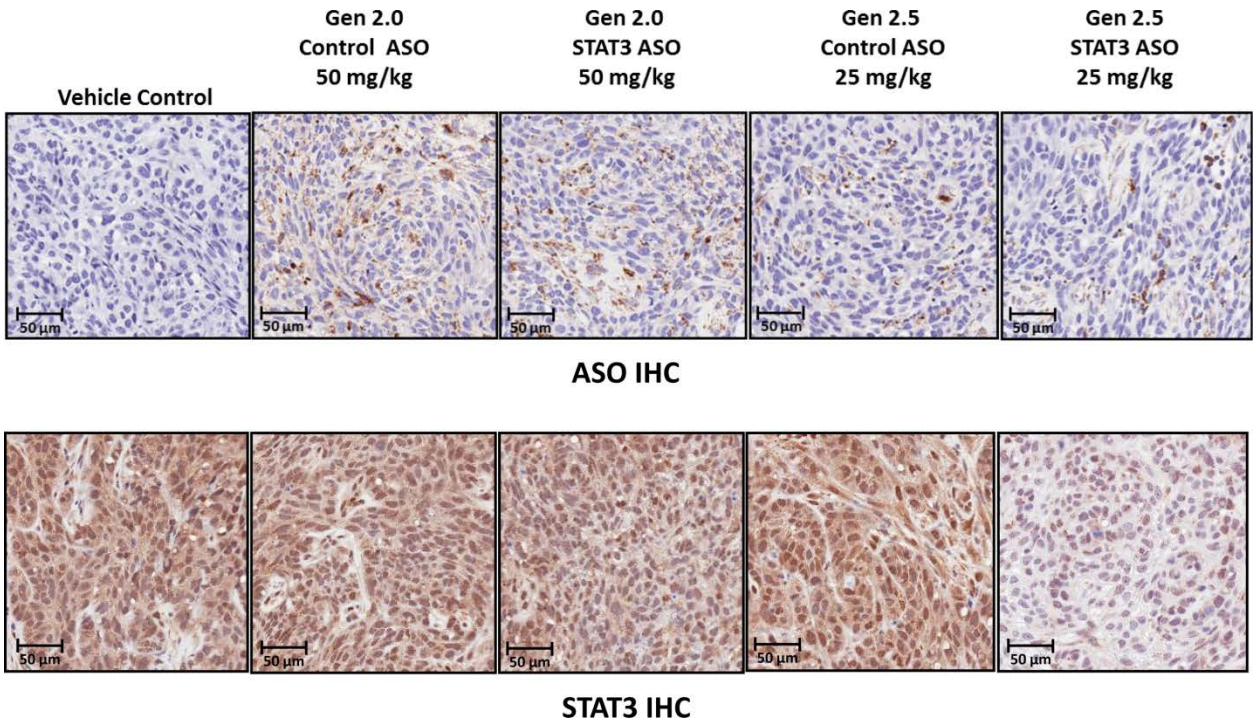


Fig. S9. IHC of ASOs and STAT3 in A431 xenograft tumors. Animals bearing A431 human epidermoid tumors in the flank region were treated with Gen 2.0 STAT3 ASO or cEt (Gen 2.5) STAT3 ASO at either 50 mg/kg (for Gen 2.0 chemistry) or 25 mg/kg (for Gen 2.5 chemistry), 5 times per week for 3 weeks as described in **Fig. 2**. Whereas the accumulation of ASOs in both A431 tumor and tumor-associated stromal cells was observed in all ASO-treated groups by IHC with an ASO-specific antibody (*top panel*), strong reduction in STAT3 was observed only in the Gen 2.5 STAT3 ASO-treated group (*bottom panel*). Scale bars, 50 µm.

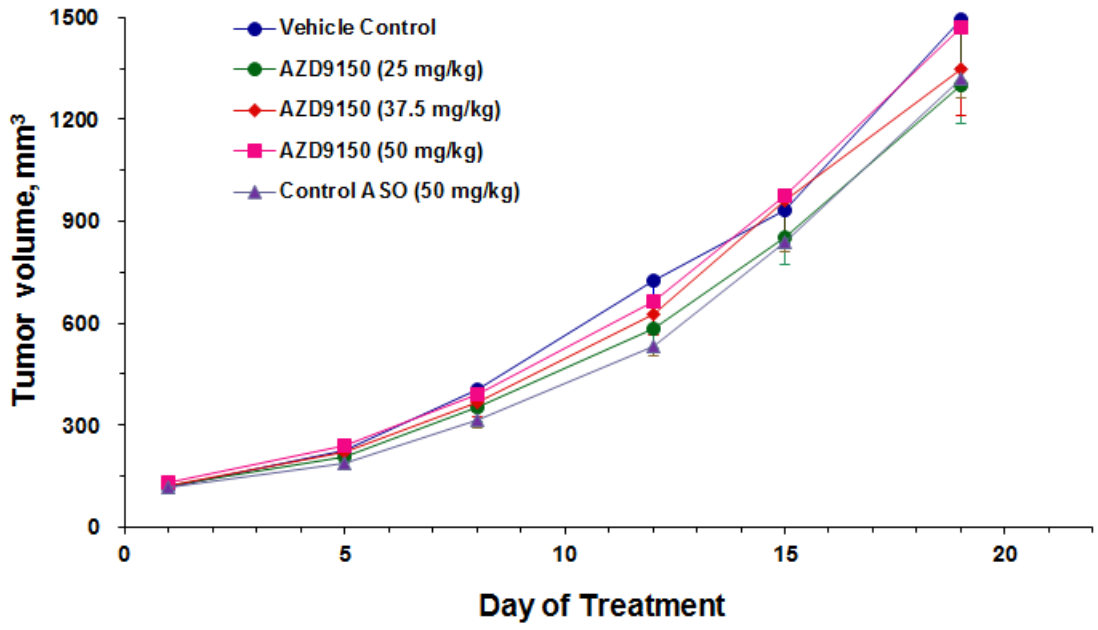


Fig. S10. Effect of AZD9150 on the growth of A431 xenograft tumors. Animals bearing A431 cells in the flank region were treated with AZD9150 at 25, 37.5, or 50 mg/kg along with a control ASO at 50 mg/kg, 5 times per week for 3 weeks. Graph shows the means ± SEM of n = 10.

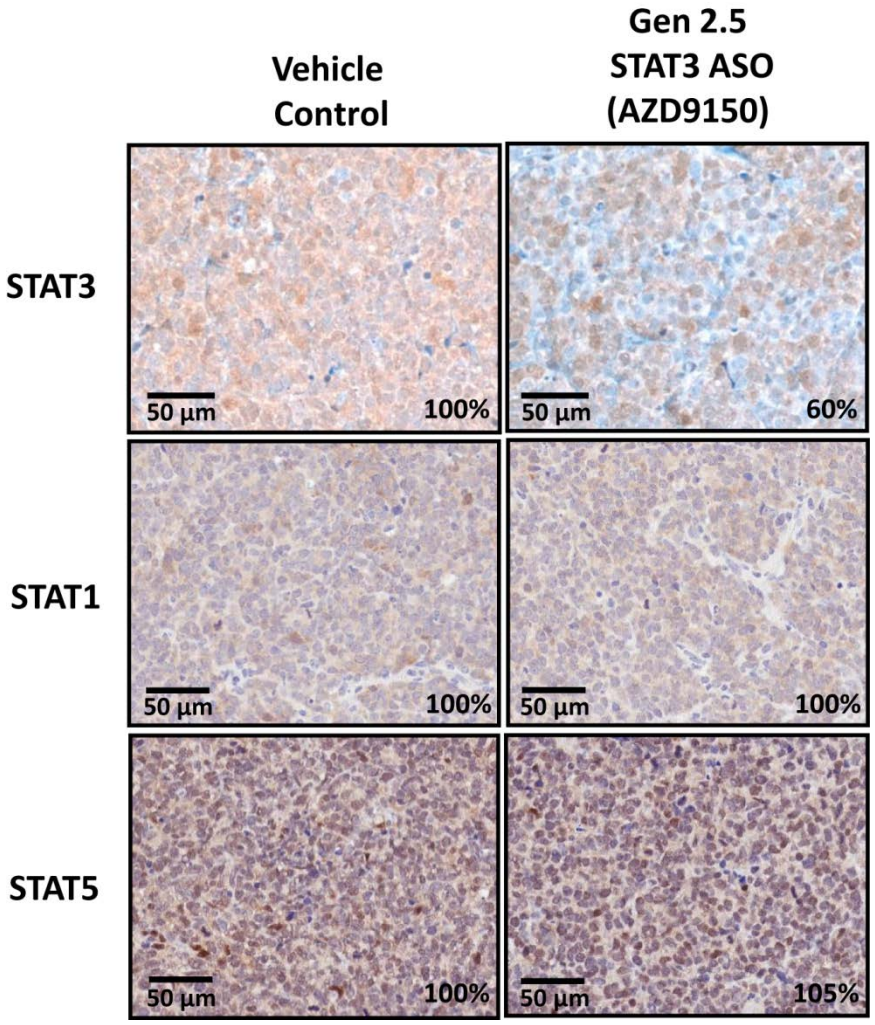


Fig. S11. Selectivity of AZD9150 for STAT3 over STAT1 or STAT5 in lymphoma patient-derived xenografts (PDX). Tumor-bearing animals were treated with AZD9150 at 50 mg/kg, daily, 5 days a week for 3 weeks, then tumors were harvested and analyzed for STAT3, STAT1, and STAT5 protein by IHC. The intensity of staining was quantified and is shown as % of vehicle control. Scale bars, 50 µm.

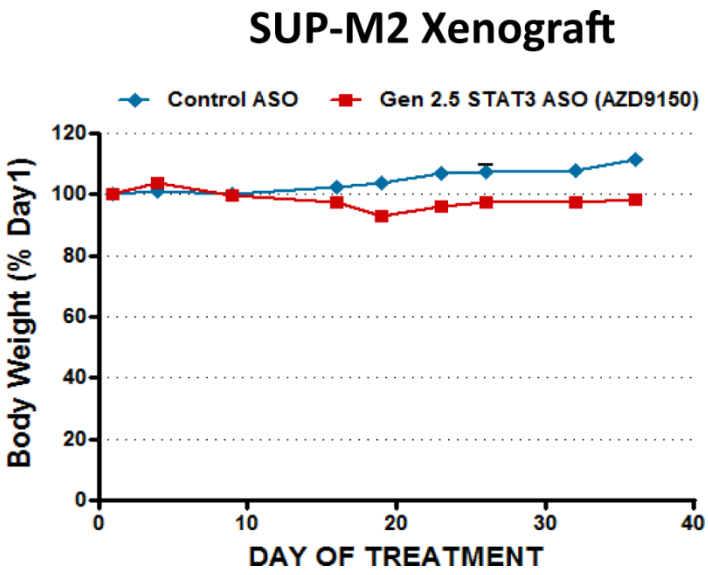


Fig. S12. Toxicity testing of AZD9150 in animals bearing SUP-M2 tumors. Systemic delivery of cEt (Gen 2.5) STAT3 ASO (AZD9150) in SUP-M2 xenograft model did not cause any significant body weight loss during the course of treatment compared to control ASO. Graph shows the means ± SEM (n=16 mice per group).

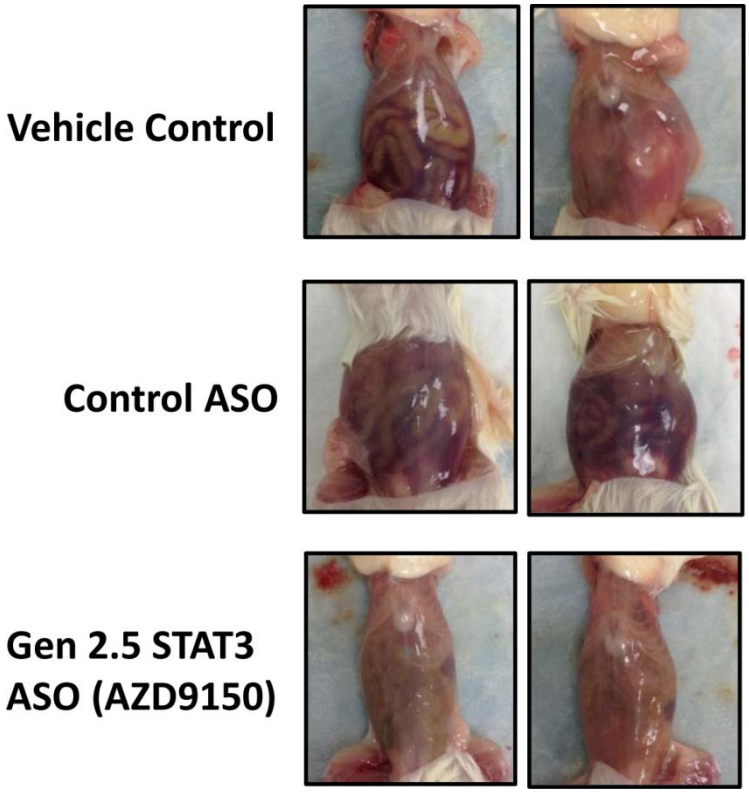


Fig. S13. Antitumor activity of cEt (Gen 2.5) STAT3 ASO (AZD9150) in SUP-M2 dissemination model. SUP-M2 cells were intravenously injected into NSG mice. Twenty days later, animals were randomized based on soluble CD30 (sCD30) concentrations and treated with either control ASO or Gen 2.5 STAT3 ASO (AZD9150) at 50 mg/kg, daily for 2 weeks. AZD9150 markedly reduced tumor burden, with much less ascites in the peritoneal cavity compared to animals treated with vehicle or control ASO.

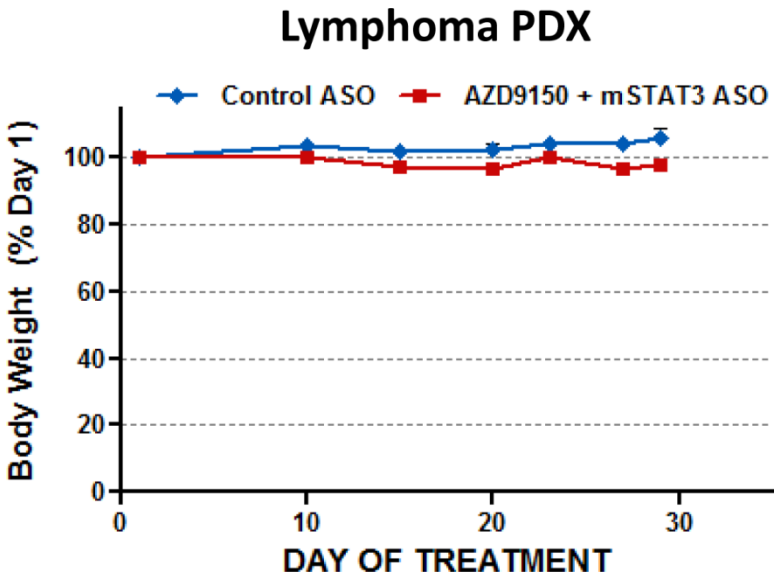
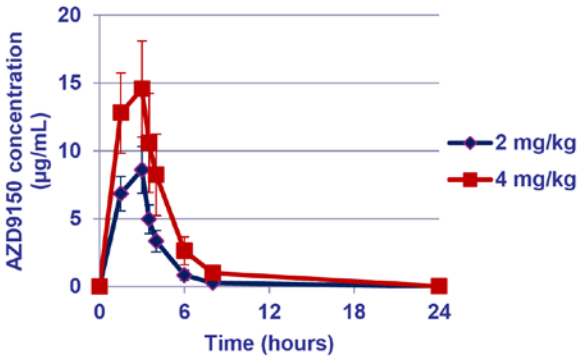
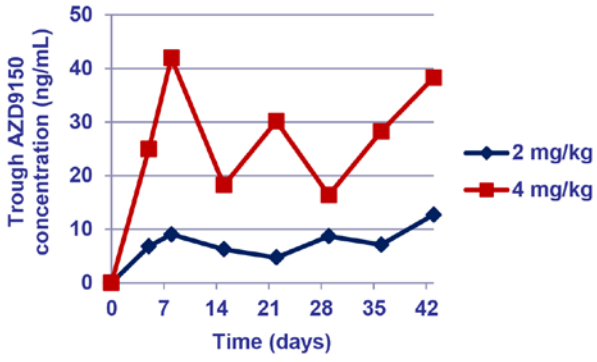


Fig. S14. Toxicity testing of AZD9150 in animals bearing patient-derived lymphoma xenografts. Systemic delivery of cEt (Gen 2.5) STAT3 ASO (AZD9150) in combination with mouse STAT3 ASO in lymphoma PDX xenograft models did not cause any significant body weight loss during the course of treatment compared to control ASO. Graph shows the means ± SEM (n=8 mice for control ASO and n=7 mice for STAT3 ASO).





Cohort	Dose Level (mg/kg)	Cycle, Day	N	C_{max} (µg/mL)	T_{max} (hr)[a]	$AUC_{0\text{-}48hr}$ (hr*µg/mL)	CL_p(L/hr)[b]
1	2	C0, D1	4	8.6 (1.72)	3 (3 – 3)	30 (7.02)	4.91 (1.39)
		C1, D15	1	6.37 (0)	3 (3 – 3)	20 (0)	7.02 (0)
2	4	C0, D1	10	15.1 (3.5)	3 (1.5 – 3)	64.4 (17.4)	4.65 (1.23)
		C1, D15	4	18 (3.5)	3 (3 – 3)	79.2 (12.7)	3.62 (0.686)

Fig. S15. Pharmacokinetic data for AZD9150. Plasma concentration of AZD9150 was determined during the course of the clinical study using a validated high performance chromatography-UV method. Data presented are mean (SD) except T_{max} (hr)[a], which is presented by median (range). CL_p(L/hr)[b](plasma clearance) = Dose (mg/kg) / $AUC_{0\text{-}48hr}$ (hr* g/mL) x 70 kg.

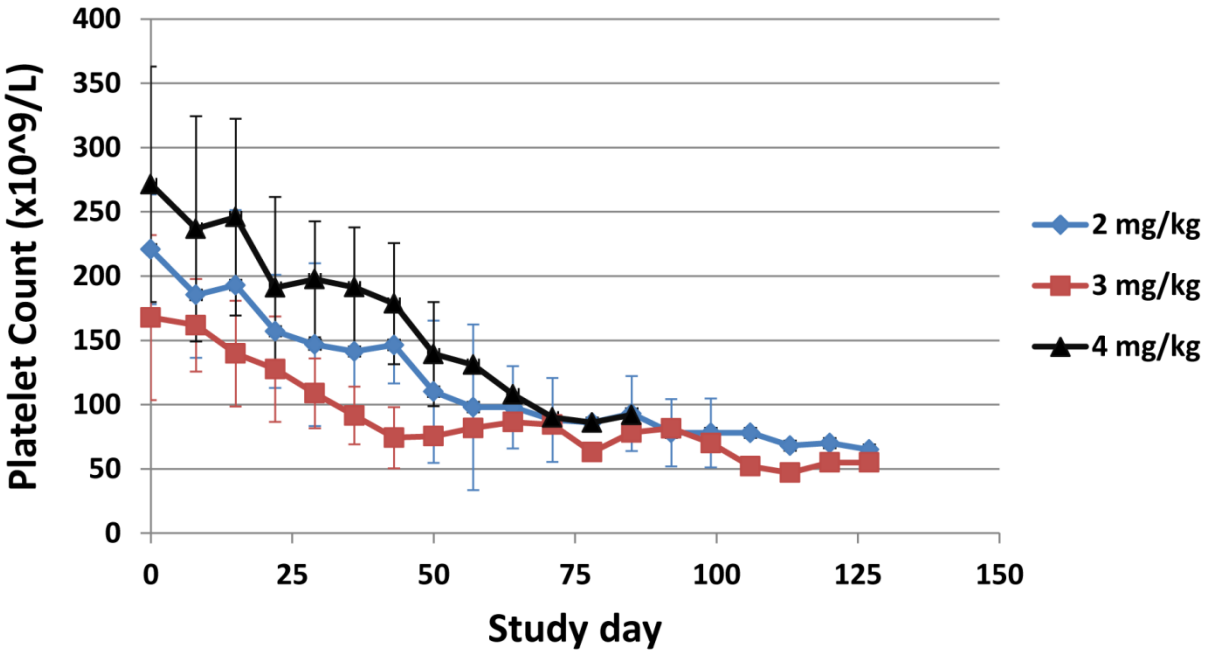


Fig. S16. Changes in platelet count. Platelets were counted at different time points in all 25 patients during the course of treatment.

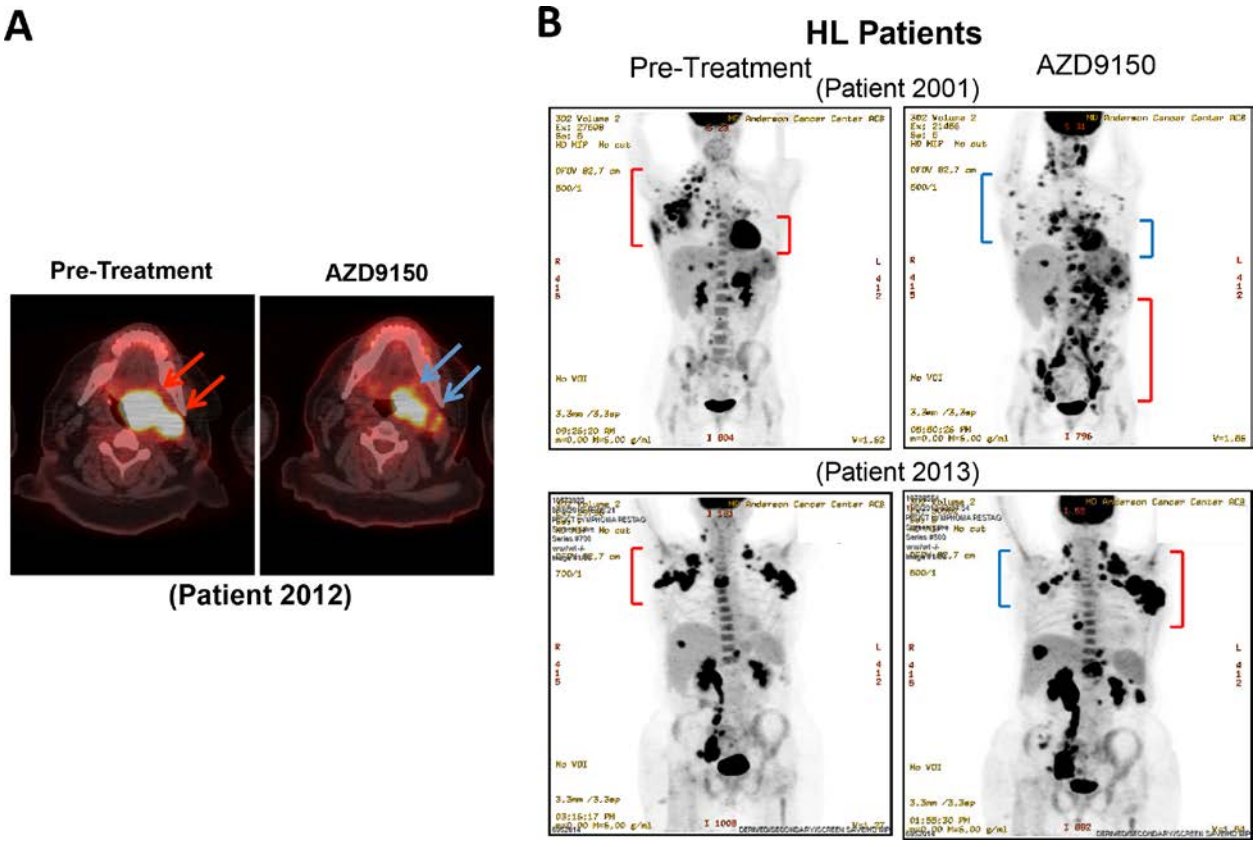


Fig. S17. Clinical antitumor activity of AZD9150. (**A**) Transverse magnetic resonance imaging scan of patient 2012, showing a large oropharyngeal mass at baseline (*left panel*) and the reduction of this mass after AZD9150 treatment (*right panel*). (**B**) PET scans for HL patients 2001 and 2013 at baseline and approximately 8 weeks after AZD9150 (2 mg/kg on study day 1, 3, and 5, and then weekly from study day 8) treatment. Red brackets or arrows indicate regions of highly metabolically active tumors, whereas blue brackets or arrows indicate regions where tumors have regressed.

Supplementary Table S1. Quantitation of ASO accumulation in A431 tumor

ASO	Dose	ASO concentration in tumor (μg/g) (n=6/group)
Gen 2.0 STAT3	50 mg/kg	98.9 ± 21.4
Gen 2.5 STAT3 (AZD9150)	25 mg/kg	59.8 ± 15.1

Supplementary Table S2. AZD9150 phase I patient demographics

	All doses (n=25)	2 mg/kg (n=4)	3 mg/kg (n=12)	4 mg/kg (n=9)
Sex				
Male	14	1	7	6
Female	11	3	2	6
Age				
Median	63	54	63	69
Range	27 to 85	45 to 63	28 to 84	27 to 85
Previous chemotherapy regimens				
Median	5	7	3	6
Range	2 to 11	3 to 10	2 to 11	2 to 10
Cancer type				
Adenoid cystic carcinoma	1	0	1	0
Breast	1	0	1	0
Colorectal	3	2	0	1
DLBCL	7	1	3	3
Follicular NHL	2	0	2	0
Hodgkin's lymphoma	2	0	0	2
Mantle cell lymphoma	1	0	0	1
NSCLC	2	0	1	1
Ovarian	1	0	0	1
Pancreatic	2	0	1	1
Prostate	1	0	0	1
Sarcoma	1	1	0	0
Ureteral transitional cell carcinoma	1	0	0	1

Supplementary Table S3. AEs greater than 5% for all doses

	All doses (n=25)		2 mg/kg (n=4)		3 mg/kg (n=12)		4 mg/kg (n=9)	
	N	%	N	%	N	%	N	%
Alanine aminotransferase increased	11	44	0	0	3	25	8	89
Aspartate aminotransferase increased	11	44	2	50	4	33	5	56
Thrombocytopenia	10	40	2	50	2	17	6	67
Anemia	5	20	1	25	1	8	3	33
Neutropenia	4	16	2	50	0	0	2	22
Diarrhea	2	8	1	25	0	0	1	11
Haptoglobin decreased	2	8	0	0	0	0	2	22
Headache	2	8	0	0	1	8	1	11
Hypokalemia	2	8	0	0	1	8	1	11
Pyrexia	2	8	0	0	2	17	0	0
Transaminases increased	2	8	0	0	1	8	1	11

Supplementary Table S4. Prior treatment regimens of DLBCL patients 1001 and 2012

Patient 1001 prior regimens	**Best response**
rituximab/cyclophosphamide/hydroxydaunorubicin/ vincristine/prednisone (R-CHOP) + rituximab maintenance	CR
ifosfamide/carboplatin/etoposide	PD
bendamustine	PD
rituximab/cyclophosphamide/hydroxydaunorubicin/ vincristine/prednisone (R-CHOP)	PD
panobinostat/everolimus	SD
lenalidomide/rituximab	PD
MK2206 (AKT inhibitor	PD
rituximab/gemcitabine/oxaliplatin	PD
gemcitabine/protein-bound paclitaxel/bevacizumab	PD
Patient 2012 prior regimens	**Best response**
rituximab/cyclophosphamide/hydroxydaunorubicin/ vincristine/prednisone (R-CHOP)	PD
rituximab/ifosfamide/carboplatin/etoposide (R-ICE)	PD

Supplementary Table S5. List of primer and probe sequences used for qRT-PCR

Gene	Forward Primer	Reverse Primer	Probe
Human *STAT3*	5'-ACATGCCACTTTGGTGTTTCATAA-3'	5'-TCTTCGTAGATTGTGCTGATAGAGAAC-3'	5'-FAM-CAGTATAGCCGCTTCCTGCAAGAGTCGAA-TAMRA-3'
Human *MCL-1*	5'-AAGATCTGGTTACGGTAACTAAAAAAGC-3'	5'-GGGCCCCTAAAAACCAATTC-3'	5'-FAM-TGTCTGCCAAATCCAGTGGAAACAAGTG-TAMRA-3'
Human *BCL6*	5'-CCGGAGTCGAGACATCTTGAC-3'	5'-TTCAACTGGTCTGTAAAGATGCTATAGAA-3'	5'-FAM-TTGTTGTGAGCCGTGAGCAGTTTAGAGC-TAMRA-3'
Human *CCND1*	5'-CCGAGAAGCTGTGCATCTACAC-3'	5'-AGGTTCCACTTGAGCTTGTTCAC-3'	5'-FAM-ACAACTCCATCCGGCCCGAGG-TAMRA-3'
Human *BIRC5*	5'-CACCACTTCCAGGGTTTATTCC-3'	5'-TGATCTCCTTTCCTAAGACATTGCT-3'	5'-FAM-ACCAGCCTTCCTGTGGGCCCCT-TAMRA-3'
Human *β-ACTIN*	5'-CGGACTATGACTTAGTTGCGTTACA-3'	5'-GCCATGCCAATCTCATCTTGT-3'	5'-FAM-CCTTTCTTGACAAAACCTAACTTGCGCAGA-TAMRA-3'
Mouse *Stat3*	5'-CGACAGCTTCCCCATGGA-3'	5'-ATGCCCAGTCTTGACTCTCAATC-3'	5'-FAM-CTGCGGCAGTTCCTGGCACCTT-TAMRA-3'
Mouse *Malat1*	5'-TGGGTTAGAGAAGGCGTGTACTG-3'	5'-TCAGCGGCAACTGGGAAA-3'	5'-FAM-CGTTGGCACGACACCTTCAGGGACT-TAMRA-3'
Mouse *Cyclophilin A*	5'-TCGCCGCTTGCTGCA-3'	5'-ATCGGCCGTGATGTCGA-3'	5'-FAM-CCATGGTCAACCCCACCGTGTTC-TAMRA-3'

Supplementary Table 6. Changes in tumor volume during the course of ASO treatment

SUP-M2

Animal ID (Vehicle)	Day 1			Day 4			Day 9			Day 16			Day 19			Day 23			Day 26			Day 32			Day 36		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
33	146	7.74	6.15	123	7.41	5.77	129	7.3	5.95	114	7.63	5.47	145	8.37	5.88	152	9.04	5.79	302	10.65	7.53	518	11.98	9.3	620	13.76	9.49
34	49	5.28	4.31	99	4.96	8.01	121	8.22	5.42	346	10.58	8.09	661	12.22	10.4	1549	16.1	13.87	2830	19.31	17.12	3655	20.51	18.88	3686	18.6	21.31
35	104	5.64	6.51	92	6.76	5.21	80	6.09	5.12	48	4.68	4.54	47	4.51	4.58	30	4.49	3.64	37	4.56	4.02	31	4.37	3.74			
36	213	8.89	6.93	199	8.41	6.88	237	8.6	7.42	219	8.62	7.13	286	10.15	7.51	356	12.15	7.65	594	12.61	9.71	1085	15.3	11.91	1504	16.14	13.65
37	131	7.18	6.03	127	7.37	5.88	175	7.49	6.84	166	8.62	6.2	290	9.63	7.76	417	8.78	10.82	498	12.27	9.01	737	15.25	9.83	1066	16.06	11.52
38	209	8.37	7.07	119	6.87	5.89	156	6.83	6.76	143	8.04	5.96	210	9.37	6.7	305	10.5	7.62	551	12.21	9.5	905	14.96	11	1250	15.43	12.73
39	123	6.71	6.05	125	6.14	6.63	165	7.06	6.83	237	8.35	7.53	276	8.14	8.33	429	10.75	8.93	493	9.81	10.24	898	12.43	12.02	1744	16.16	14.69
40	164	7.35	6.68	175	7.36	6.9	170	7.64	6.68	203	8.09	7.09	248	8.73	7.54	431	10.72	8.97	508	11.58	9.37	921	14.28	11.36	1334	15.64	13.06
41	143	7.55	6.16	119	7.39	5.68	147	6.77	6.59	29	4.73	3.49	21	3.66	3.36	0			0			0			0		
42	123	6.95	5.95	135	6.72	6.33	116	6.47	5.98	30	4.18	3.77	33	4.07	4.01	0			0			0			0		
43	165	6.37	8.12	119	7.12	5.78	125	6.46	6.22	113	6.34	5.96	242	7.64	8.29	294	9.08	8.05	414	10.1	9.05	930	13.87	11.58	1060	14.58	12.06
44	133	5.97	7.49	116	5.67	7.2	160	6.32	8.03	114	8.14	5.29	239	9.49	7.09	369	10.39	8.43	540	10.96	9.93	661	14.52	9.54	887	12.63	11.85
45	135	6.74	6.32	95	5.73	5.79	81	5.45	5.48	63	4.68	5.72	119	6.41	6.1	83	5.49	5.52		0			0				
46	96	5.67	5.97	175	8.09	6.58	161	6.99	6.79	101	6.81	5.45	168	7.08	6.88	242	8.25	7.66	282	9.38	7.76	424	10.81	8.86	614	12.08	10.08
47	152	6.88	6.65	182	7.14	7.14	200	7.23	7.65	264	8.36	7.95	317	9.15	8.33	604	12.13	9.98	920	14.39	11.31	1245	12.1	17.01	1593	17.56	13.47
48	156	7.76	6.34	137	8.27	5.75	215	8.56	7.09	209	9.64	6.59	214	10.55	6.37	308	11.79	7.23	422	12.36	8.26	506	13.19	8.76	653	15.1	9.3

Animal ID (AZD9150)	Day 1			Day 4			Day 9			Day 16			Day 19			Day 23			Day 26			Day 32			Day 36		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
65	182	7.24	7.09	151	7.76	6.23	179	8.82	6.37	169	8.11	6.46	195	8.62	6.73	383	10.72	8.45	392	10.89	8.48	523	12.34	9.21	632	13.49	9.68
66	171	7.81	6.62	126	6.45	6.25	130	6.81	6.17	72	4.98	5.78	56	4.81	4.88	46	4.6	4.48	47	4.91	4.37	40	4.16	4.6	0		
67	138	6.49	6.57	140	6.46	6.7	87	5.82	5.47	99	6.06	5.71	43	4.74	4.25	35	3.99	4.41	0			0			0		
68	128	6.18	6.72	256	7.93	8.13	316	9.45	8.18	421	10.01	9.17	455	10.54	9.29	475	10.95	9.31	833	12.53	11.53	1258	15.24	12.85	1600	15.69	14.28
69	80	5.14	6.07	116	6.12	6.17	74	5.77	5.06	51	4.78	4.64	63	5.32	4.85	65	5.73	4.77	80	5.82	5.23	103	5.69	6.34	108	6.4	5.81
70	140	6.87	6.39	149	6.92	6.56	133	6.32	6.65	166	7.38	6.7	208	7.76	7.33	283	9.13	7.88	322	8.66	8.62	547	10.29	10.34	663	12.08	10.48
71	151	7.56	6.32	148	7.86	6.13	124	7.4	5.78	71	6.28	4.77	61	6.47	4.35	79	4.99	6.37	72	5.18	5.35	156	7.24	6.57	217	8.2	7.27
72	124	5.93	7.08	197	7.21	7.57	146	7.42	6.28	126	6.9	6.04	166	8.04	6.43	280	7.37	10.3	293	10.27	7.56	369	10.71	8.3	757	10.97	12.58
73	123	6.92	5.96	215	8.89	6.95	162	8.14	6.3	118	6.66	5.96	83	6.08	5.23	101	5.54	6.55	92	6.32	5.4	74	5.12	5.61	54	4.67	4.96
74	128	6.72	6.18	191	6.66	8.61	180	7.67	6.86	122	7.76	5.6	161	8.83	6.04	211	10.65	6.29	226	10.89	6.44	268	12.03	6.68	306	11.87	7.18
75	97	6.46	5.48	161	6.88	6.84	104	6.44	5.67	78	5.61	5.27	23	3.87	3.42	0			0			0			0		
76	84	5.59	5.48	200	7.48	7.31	535	10.01	10.67	664	12.5	10.31	884	14.94	10.88	1200	17.18	11.82	1687	17.77	13.78	2176	18.81	15.21	2803	20.49	16.54
77	80	5.34	5.62	53	5.12	4.56	47	4.73	4.47	0			0			0			0			0			0		
78	138	8.16	5.82	182	8.1	6.7	143	7.39	6.22	126	6.58	6.19	103	6.42	5.67	87	5.39	6.02	105	6.55	5.66	82	5.44	5.52	75	5.48	5.24
79	206	7.62	7.36	173	9.25	6.11	108	7.96	5.22	127	7.02	6.02	90	6.26	5.37	67	5.72	4.83	38	4.26	4.2	66	6.34	4.58	42	5.24	4.02
80	86	5.45	5.76	112	6.65	5.8	106	6.02	5.93	84	5.5	5.55	45	4.65	4.42	53	5.01	4.58	39	4.48	4.19	51	4.56	4.95	30	3.91	3.99

Supplementary Table 6 (cont'd). Changes in tumor volume during the course of ASO treatment

Lymphoma PDX

Animal Id (Control ASO)	Day 1			Day 10			Day 15			Day 20			Day 23			Day 27			Day 29		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
011	113	7.55	5.37	170	7.4	6.65	616	16.75	8.41	890	17.9	9.78	1098	18.51	10.68	1126	18.91	10.7	1713	19.76	12.91
015	94	5.79	5.6	99	5.8	5.72	67	5.61	4.79	48	5.53	4.09	85	8.21	4.45	134	10.22	5.02	190	10.89	5.8
028	147	7.33	6.2	358	9.19	8.65	435	10.9	8.76	367	10.33	8.27	450	11.04	8.85	573	10.66	10.17	651	12.7	9.93
044	167	7.54	6.53	286	9.74	7.52	632	16.7	8.53	692	15.96	9.13	1029	18.3	10.4	1062	16.78	11.03	1604	19.22	12.67
046	128	6.45	6.18	160	6.9	6.68	192	7.88	6.84	306	8.68	8.24	358	9.09	8.7	433	9.68	9.27	671	12.68	10.09
074	153	9.34	5.62	432	11.85	8.37	597	13.25	9.31	801	14.75	10.22	920	15.12	10.82	1314	17.17	12.13	1412	17.18	12.57
075	117	6.24	6.01	342	11.81	7.46	467	13.3	8.22	635	13.78	9.41	699	14.17	9.74	934	16.33	10.49	1103	17.1	11.14
078	106	6.59	5.56	221	14.23	5.47	382	15.28	6.93	463	14.11	7.94	741	17.83	8.94	688	16.22	9.03	1239	17.65	11.62

Animal Id (AZD9150 + mSTAT3 ASO)	Day 1			Day 10			Day 15			Day 20			Day 23			Day 27			Day 29		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
003	115	7.86	5.31	167	8.2	6.26	215	9.45	6.62	509	13.52	8.51	470	11.55	8.85	410	10.62	8.62	438	12.17	8.32
017	140	7.39	6.03	157	7.51	6.35	295	14.63	6.23	412	16.54	6.92	529	16.8	7.78	615	17.43	8.24	492	17.03	7.45
026	154	7.39	6.33	456	15.1	7.62	530	14.64	8.34	650	14.76	9.2	732	15.24	9.61	807	16.42	9.72	909	17.48	10
040	77	5.39	5.25	212	10.1	6.35	315	13.96	6.59	372	13.27	7.34	352	13.66	7.04	349	14.48	6.81	365	14.21	7.03
041	137	7	6.14	232	8.77	7.14	238	11.16	6.41	288	11.13	7.06	333	11.6	7.43	304	12.27	6.9	284	11.32	6.95
063	102	5.9	5.77	158	6.95	6.61	154	6.73	6.63	199	8.33	6.78	191	8.44	6.6	257	8.5	7.63	261	9.2	7.38
076	119	6.49	5.95	390	9.24	9.01	550	11.53	9.58	696	12.01	10.56	756	11.99	11.01	840	12.09	11.56	843	12.09	11.58

PC-9

Animal Id (Vehicle)	Day 1			Day 5			Day 12			Day 19			Day 26			Day 32		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
004	98	5.97	5.63	127	6.98	5.92	138	6.48	6.41	144	6.86	6.35	217	7.62	7.4	292	8.34	8.21
019	124	7	5.84	142	6.73	6.37	173	7.57	6.63	416	9.94	8.97	477	10	9.58	528	10.51	9.83
029	103	6.95	5.33	129	7.76	5.65	149	6.95	6.42	271	8.66	7.76	142	8.37	5.71	118	7.87	5.38
052	117	8.49	5.15	144	9.99	5.27	309	10.78	7.42	389	13.15	7.54	340	12.45	7.25	355	11.35	7.76
062	170	8.55	6.19	310	10.27	7.62	499	10.73	9.46	508	11.6	9.18	495	11.5	9.1	701	11.76	10.71
068	127	9.09	5.19	180	11	5.61	172	9.07	6.04	264	11.02	6.79	255	9.16	7.32	239	11.69	6.27
097	114	6.1	5.99	98	6.32	5.46	178	8.15	6.48	291	10.85	7.18	413	11.43	8.34	784	13.29	10.65

Animal Id (AZD9150)	Day 1			Day 5			Day 12			Day 19			Day 26			Day 32		
	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)	Vol(mm3)	L(mm)	W(mm)
020	135	7.43	5.92	205	7.43	7.28	130	6.82	6.06	115	7.27	5.52	146	7.04	6.31	111	6.52	5.71
021	105	8.5	4.87	94	7.43	4.94	94	6.42	5.3	94	7.13	5.04	82	6.13	5.06	91	6.22	5.3
051	145	8.25	5.81	227	9.89	6.64	150	6.94	6.44	114	6.7	5.72	182	7.76	6.72	165	8.07	6.28
069	153	7.82	6.13	153	7.71	6.18	140	7.35	6.06	84	6.35	5.03	77	6.68	4.71	70	5.85	4.78
070	155	10.13	5.42	180	9.81	5.94	192	8.95	6.43	239	9.83	6.84	229	10.15	6.59	285	10.57	7.2
074	114	7.21	5.51	98	6.58	5.36	208	8.18	6.99	232	8.72	7.16	296	9.56	7.71	366	10.5	8.19
094	130	7.2	5.89	189	8.45	6.56	327	10.04	7.92	220	9.09	6.82	225	8.37	7.19	194	8.58	6.59