Children's Hospital Boston
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Universita' di Torino
Dipartimento di Biotecnologie Molecolari
e Scienze della Salute

ALK+ lymphomas and beyond

Roberto Chiarle, M.D.

1st CUNEO CITY IMMUNOTHERAPY CONFERENCE (CCITC) –
IMMUNOTHERAPY IN HEMATOLOGICAL MALIGNANCIES 2018
Cuneo, 17-19 maggio 2018

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DICHIARAZIONE

Relatore: Roberto Chiarle

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(Pfizer Inc.)**
- Partecipazione ad Advisory Board **(NIENTE DA DICHIARARE)**
- Titolarietà di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(Vedantra. Inc.)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**

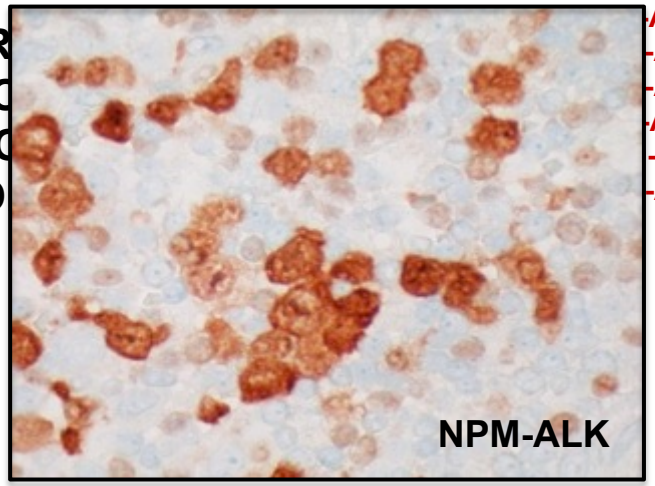
The Anaplastic Lymphoma Kinase (ALK) oncogene

ALK POSITIVE TUMORS (ALKomas)

FULL-LENGTH ALK

OVEREXPRESSED
MUTATED

NEUR
GLIC
RHABDC
THYRO



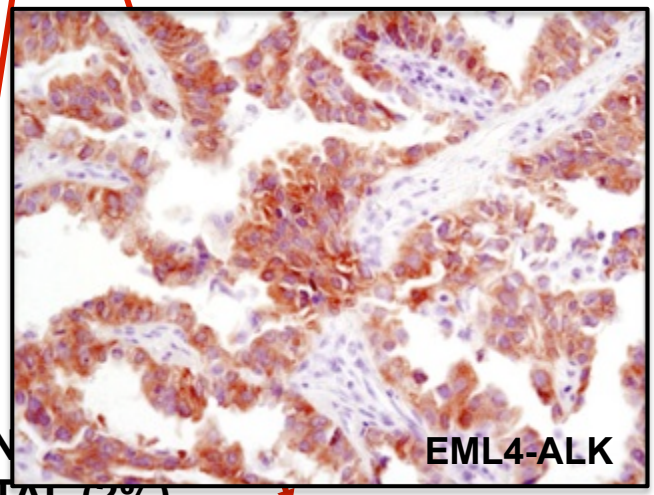
ALK CHIMERIC PROTEINS

ANAPLASTIC LARGE CELL LYMPHOMA (60%)

NPM-ALK
ALO17-ALK
TFG-ALK
-ALK
-ALK
-ALK
-ALK
-ALK
-ALK

NSCLC
BREAST CAN
COLORECTAL (2%)

EML4-ALK
KIF5B-ALK
C2orf44-ALK
TFG-ALK
HIP1-ALK
KLC1-ALK
PTPT3-ALK
TRK-ALK



RENAL CELL CARCINOMA

VCL-ALK

Chiarle et al, Nature Rev Cancer 2008
Hallberg and Palmer, Nature Rev Cancer 2013

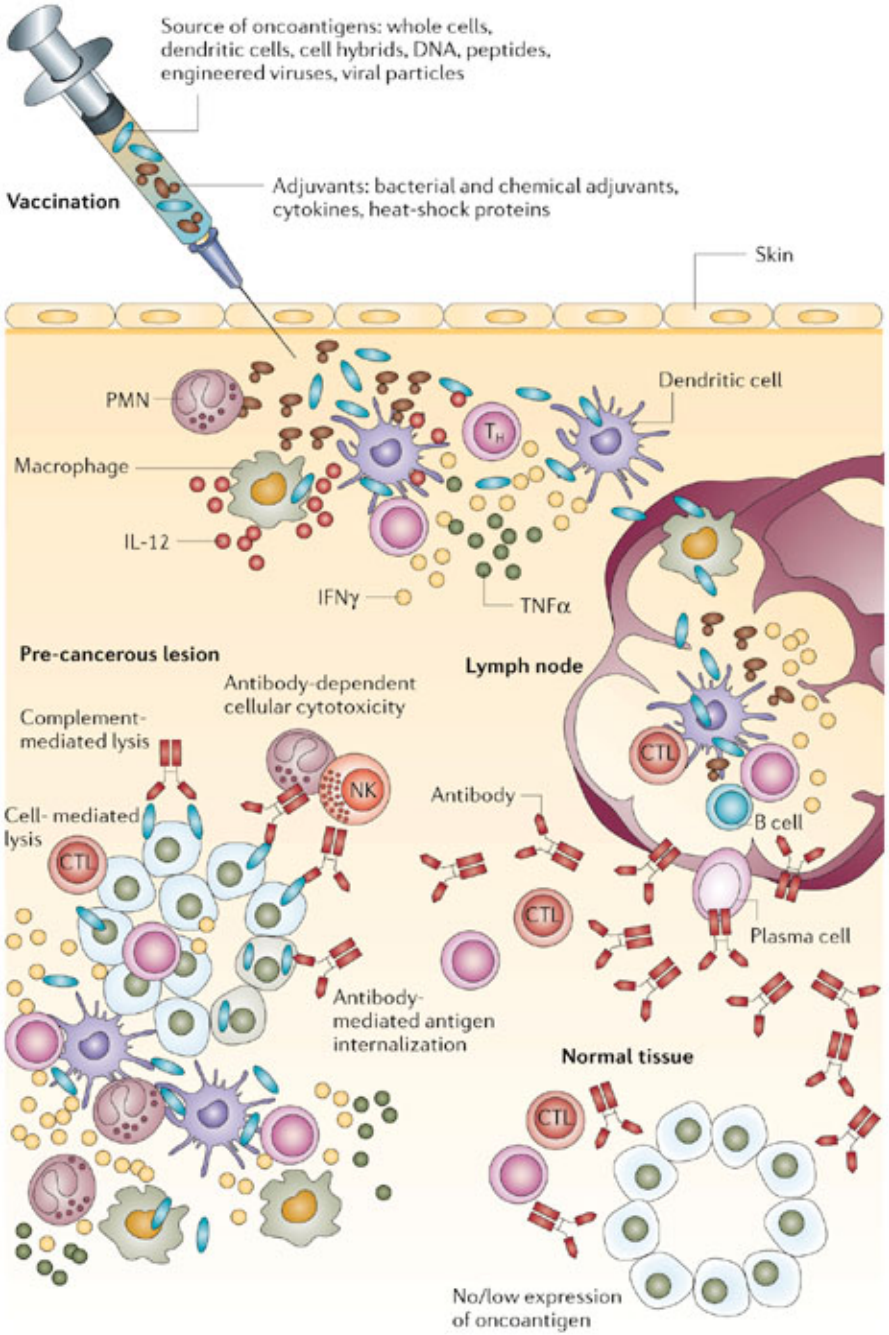
Key immunological properties of ALK as an oncoantigen

- Restricted and low levels of expression in normal tissues
- Selective and high expression by the tumor cells
- Strong addiction of the neoplastic cells on the ALK signalling for their growth and survival
- Good spontaneous immunogenicity of the ALK protein in human patients

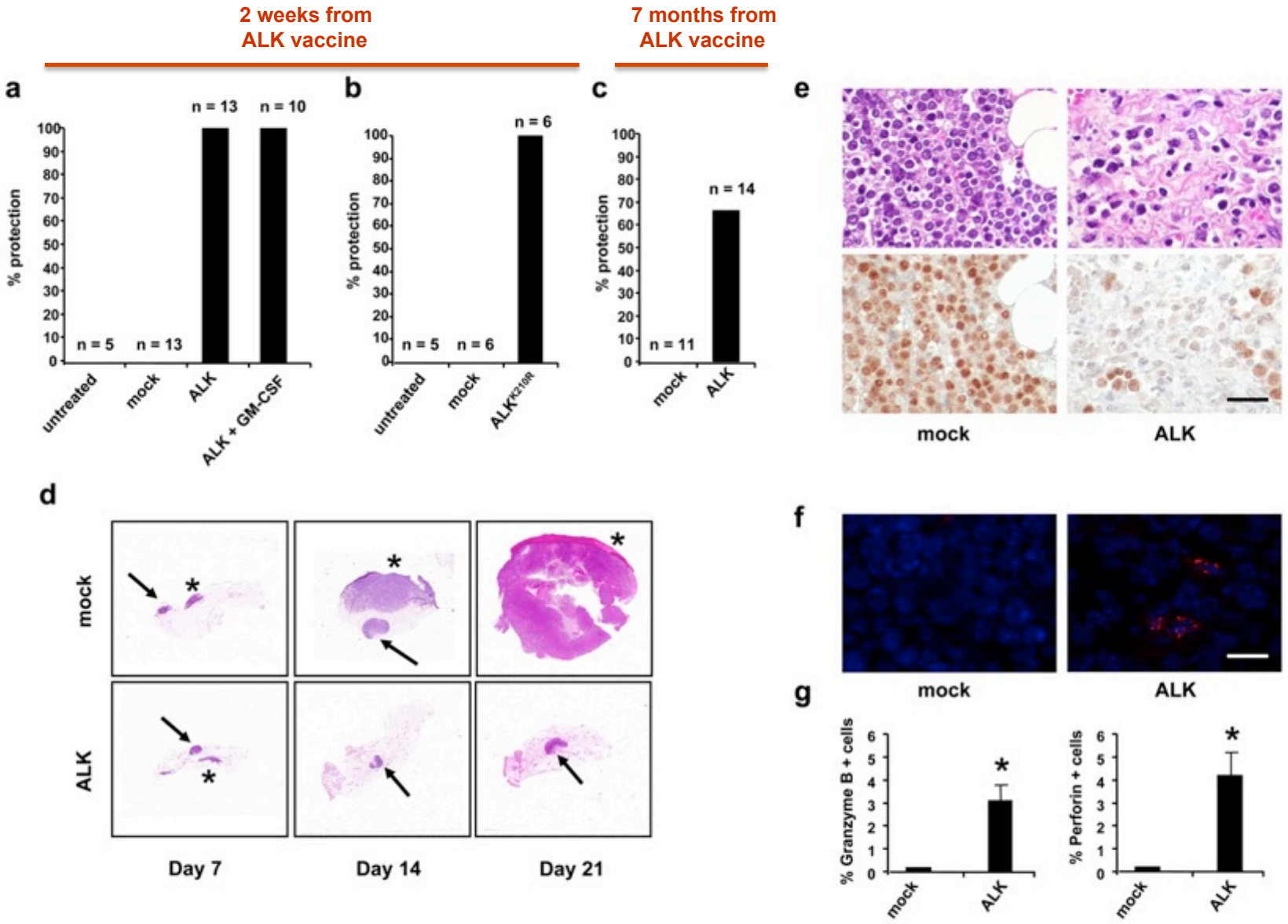
Spontaneous anti-ALK immune response in patients with ALK-rearranged tumors

- Circulating antibodies against NPM-ALK protein are found in ALK-positive ALCL patients (*Pulford K et al, Blood 2000; Mussolin et al, Leukemia 2009*) and are associated with better prognosis (*Ait-Tahar K et al, Blood 2010)*
- Effector and memory T CD8+ lymphocytes are present in patients with ALK lymphomas (*Passoni et al. Blood 2002; Passoni et al. Haematologica 2006*)
- CD4 T-helper responses to ALK are present in patients with ALK+ ALCL (*Ait-Tahar K et al, Cancer Res 2007*)
- Combination of minimal disseminated disease (MDD) and antibody titers can stratify ALK+ ALCL into prognostic groups (*Mussolin et al, Leukemia 2013*)
- Anti-ALK antibodies in ALK-rearranged NSCLC (*Awad et al, Oncotarget 2017)*

ENHANCING TUMOR IMMUNITY BY ALK VACCINATION

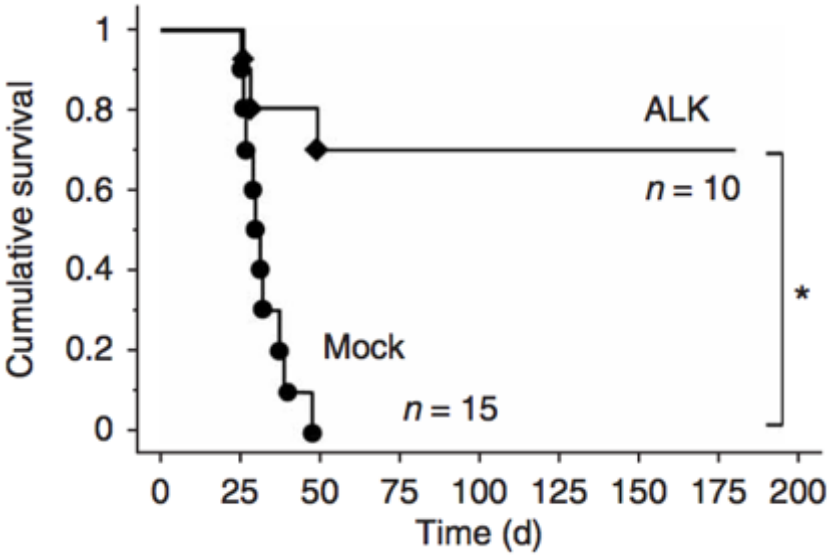


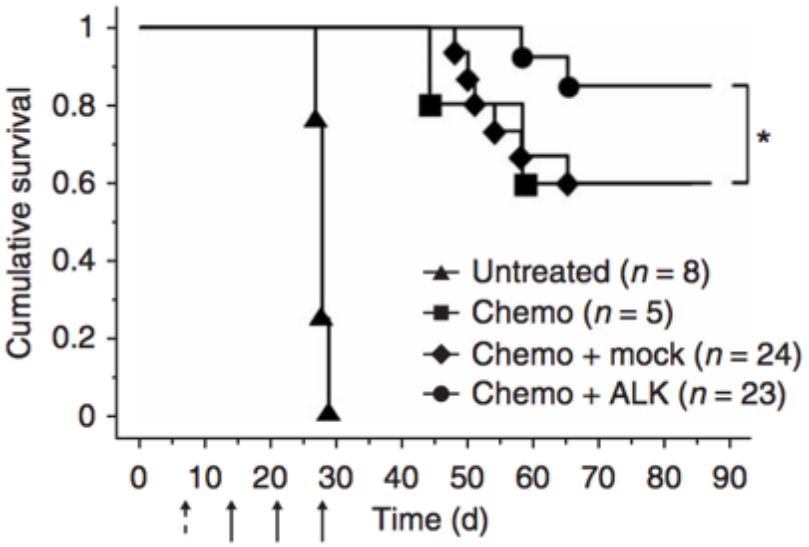
ALK DNA vaccine prevents lymphoma growth



Chiarle et al, *Nat Med* 2008

ALK DNA vaccine as therapy against lymphoma





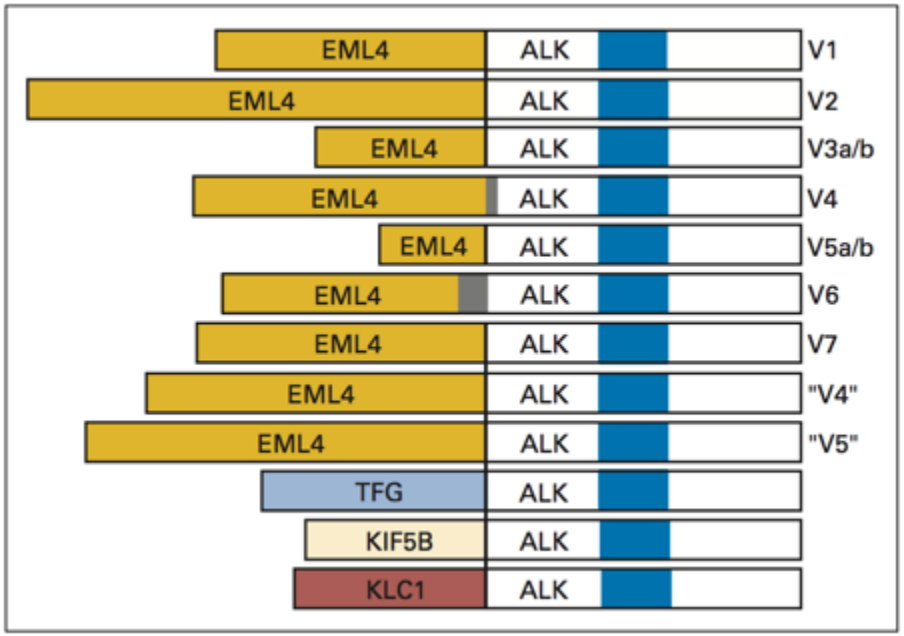
Chiarle et al, *Nat Med* 2008

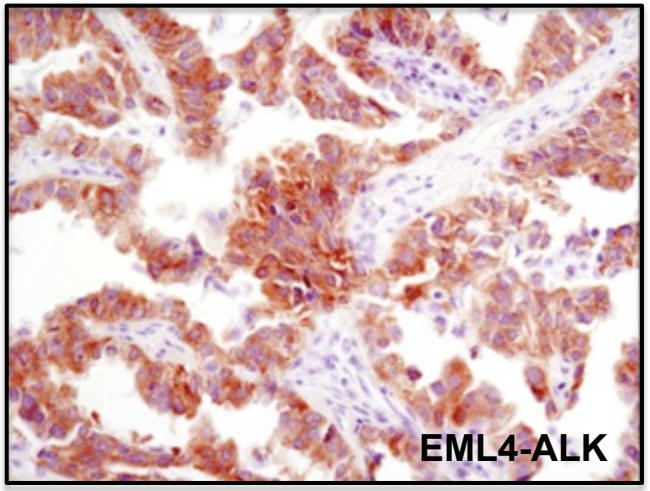
What we have learned from pre-clinical studies on ALK vaccine against ALK+ lymphoma?

- ALK vaccination elicits a specific cytotoxic immune response
- Effector CD8+ T cells and IFN-γ production, but not B cells or antibodies, are essential for the generation of a protective anti-ALK immune response
- ALK vaccination is long lasting
- The efficacy of ALK vaccination depends on the tumor burden
- ALK vaccination prevents 100% of ex-novo lymphoma growth
- ALK vaccination is synergic with chemotherapy in lymphoma treatment

ALK vaccine against solid cancers

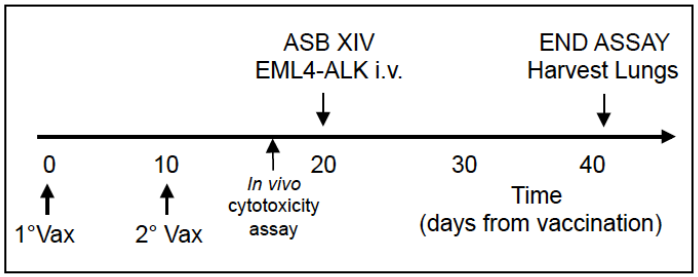
ALK-rearranged NSCLC

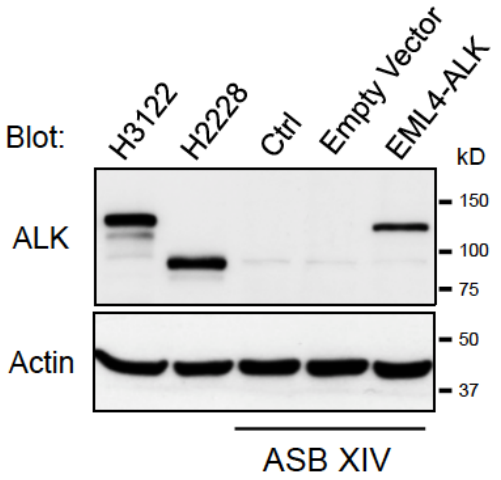


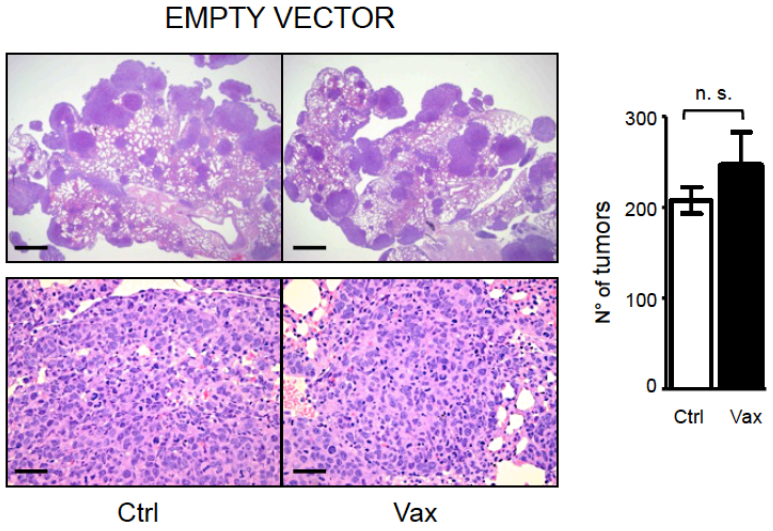


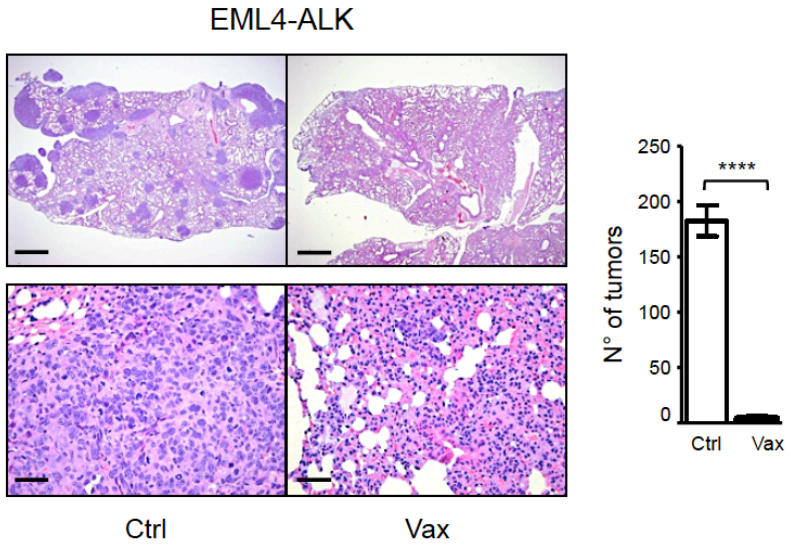
Shaw and Engelman, JCO, 2013

Prophylactic Efficacy of the ALK vaccine against lung cancer



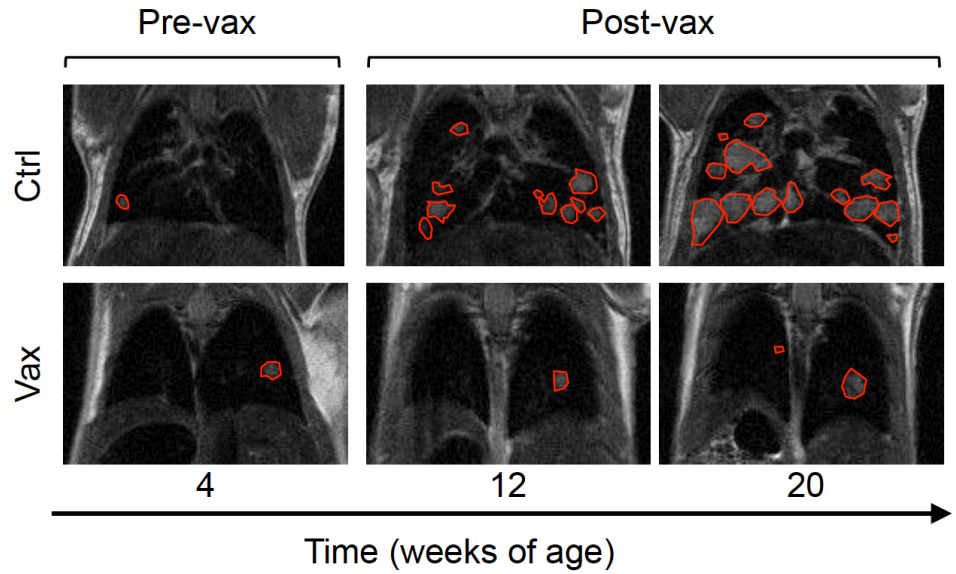


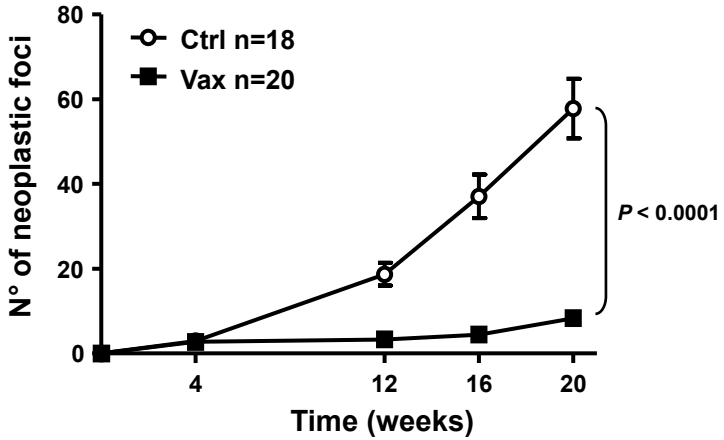


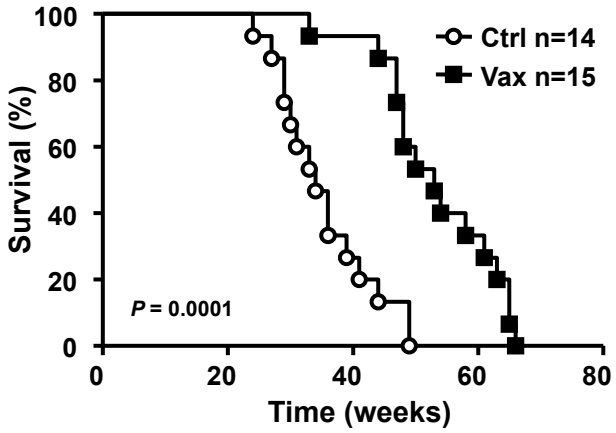


Voena et al, *Cancer Res Immunol* 2015

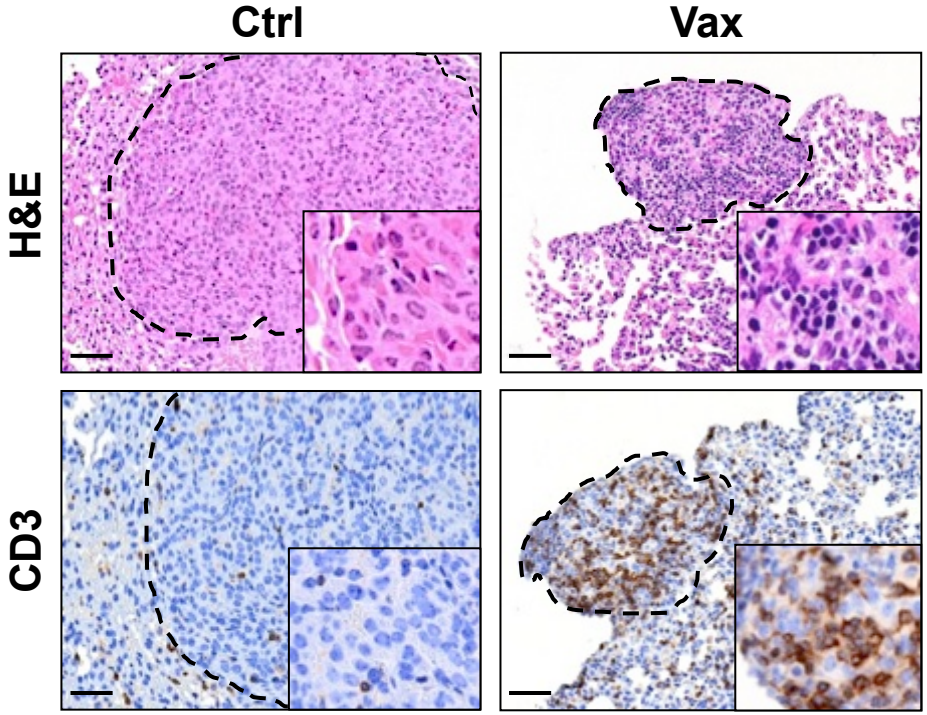
ALK vaccine significantly delays lung cancer progression and extends survival

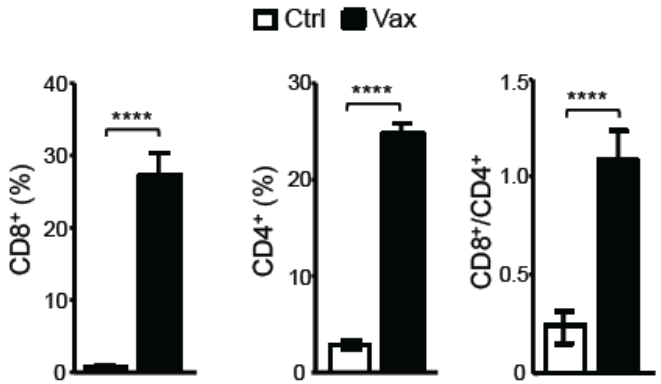






ALK vaccine increases intratumor T_{eff} cells





Potential ALK vaccine therapeutic combinations

- ALK vaccine and ALK inhibitors

- ALK vaccine and immune checkpoint blockade

Is ALK vaccine efficient in combination with ALK kinase inhibitor treatment?

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 28, 2010 VOL. 363 NO. 18

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D., Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D., Sai-Hong I. Ou, M.D., Ph.D., Bruce J. Dezube, M.D., Pasi A. Jänne, M.D., Ph.D., Daniel B. Costa, M.D., Ph.D., Marileila Varella-Garcia, Ph.D., Woo-Ho Kim, M.D., Thomas J. Lynch, M.D., Panos Fidias, M.D., Hannah Stubbs, M.S., Jeffrey A. Engelman, M.D., Ph.D., Lecia V. Sequist, M.D., M.P.H., WeiWei Tan, Ph.D., Leena Gandhi, M.D., Ph.D., Mari Mino-Kenudson, M.D., Greg C. Wei, Ph.D., S. Martin Shreeve, M.D., Ph.D., Mark J. Ratain, M.D., Jeffrey Settleman, Ph.D., James G. Christensen, Ph.D., Daniel A. Haber, M.D., Ph.D., Keith Wilner, Ph.D., Ravi Salgia, M.D., Ph.D., Geoffrey I. Shapiro, M.D., Ph.D., Jeffrey W. Clark, M.D., and A. John Iafrate, M.D., Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

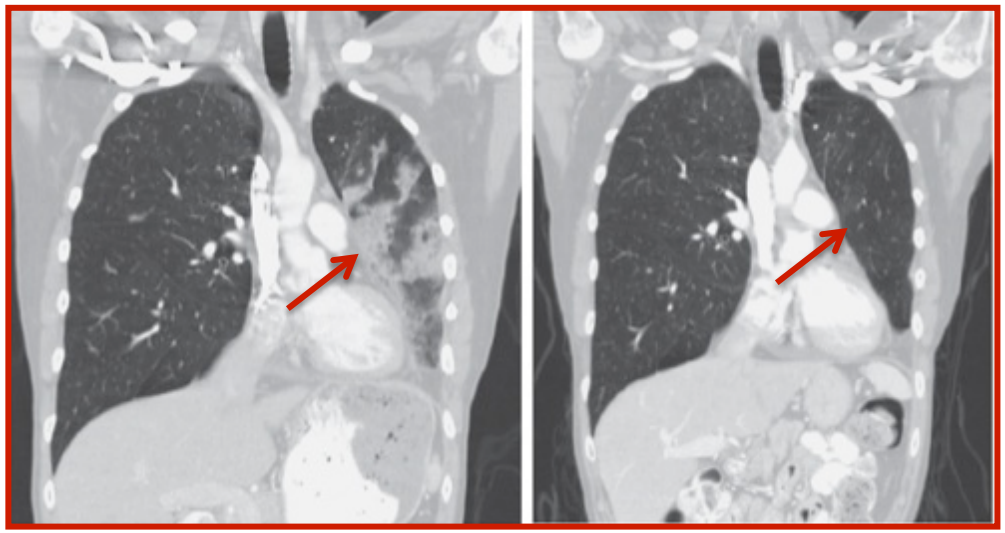
ORIGINAL ARTICLE

N ENGL J MED 368;25 NEJM.ORG JUNE 20, 2013

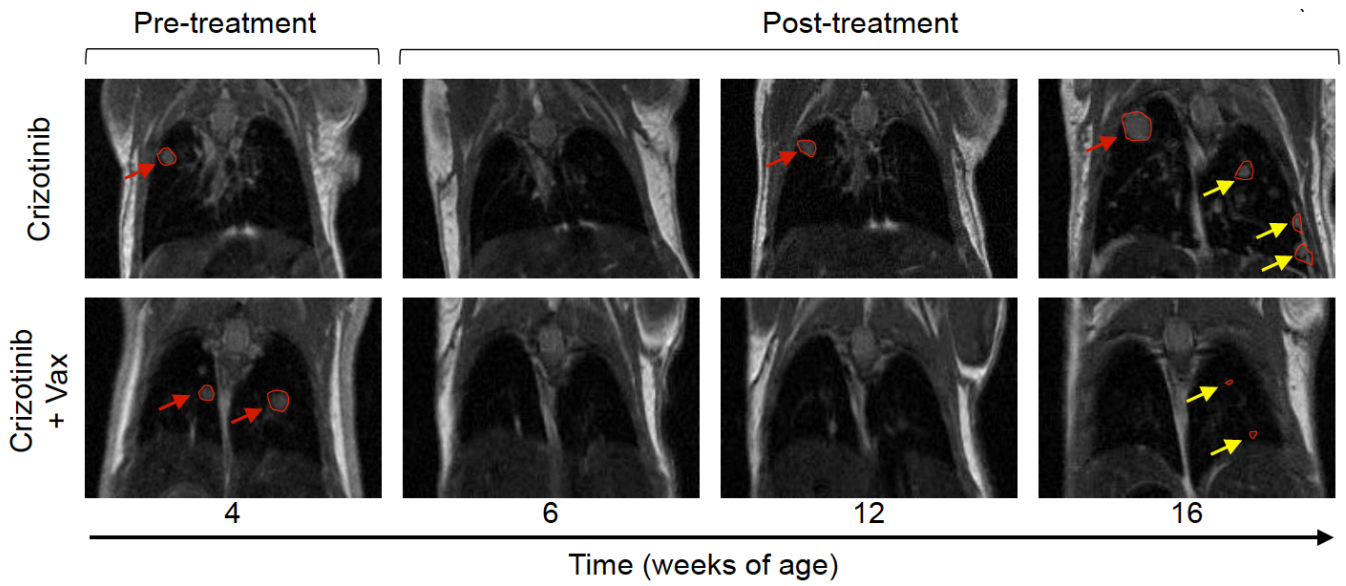
Crizotinib versus Chemotherapy in Advanced *ALK*-Positive Lung Cancer

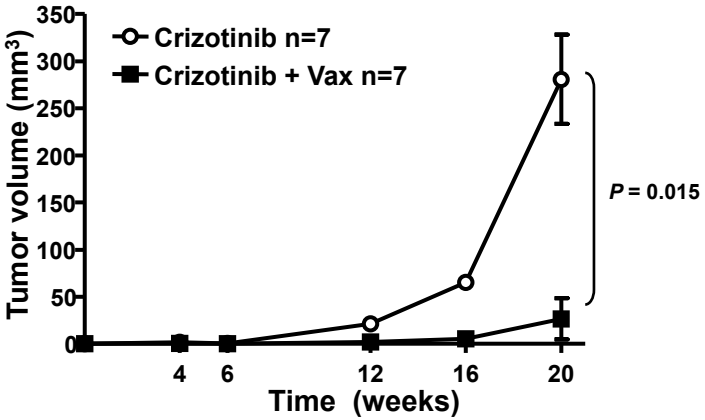
Alice T. Shaw, M.D., Ph.D., Dong-Wan Kim, M.D., Ph.D., Kazuhiko Nakagawa, M.D., Ph.D., Takashi Seto, M.D., Lucio Crinó, M.D., Myung-Ju Ahn, M.D., Tommaso De Pas, M.D., Benjamin Besse, M.D., Ph.D., Benjamin J. Solomon, M.B., B.S., Ph.D., Fiona Blackhall, M.D., Ph.D., Yi-Long Wu, M.D., Michael Thomas, M.D., Kenneth J. O'Byrne, M.D., Denis Moro-Sibilot, M.D., D. Ross Camidge, M.D., Ph.D., Tony Mok, M.D., Vera Hirsh, M.D., Gregory J. Riely, M.D., Ph.D., Shrividya Iyer, Ph.D., Vanessa Tassell, B.S., Anna Polli, B.S., Keith D. Wilner, Ph.D., and Pasi A. Jänne, M.D., Ph.D.

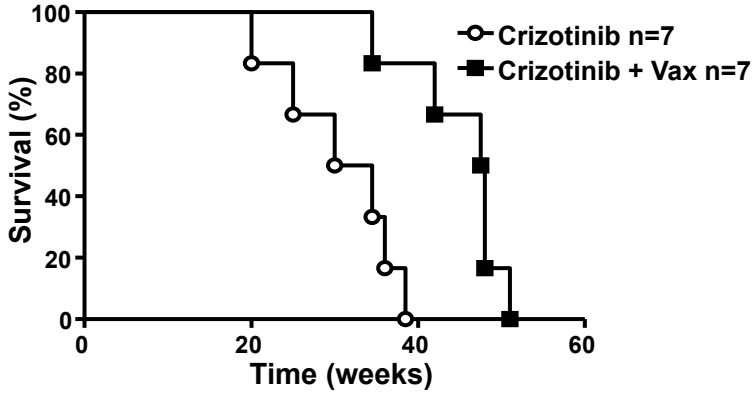
Before crizotinib **After crizotinib**



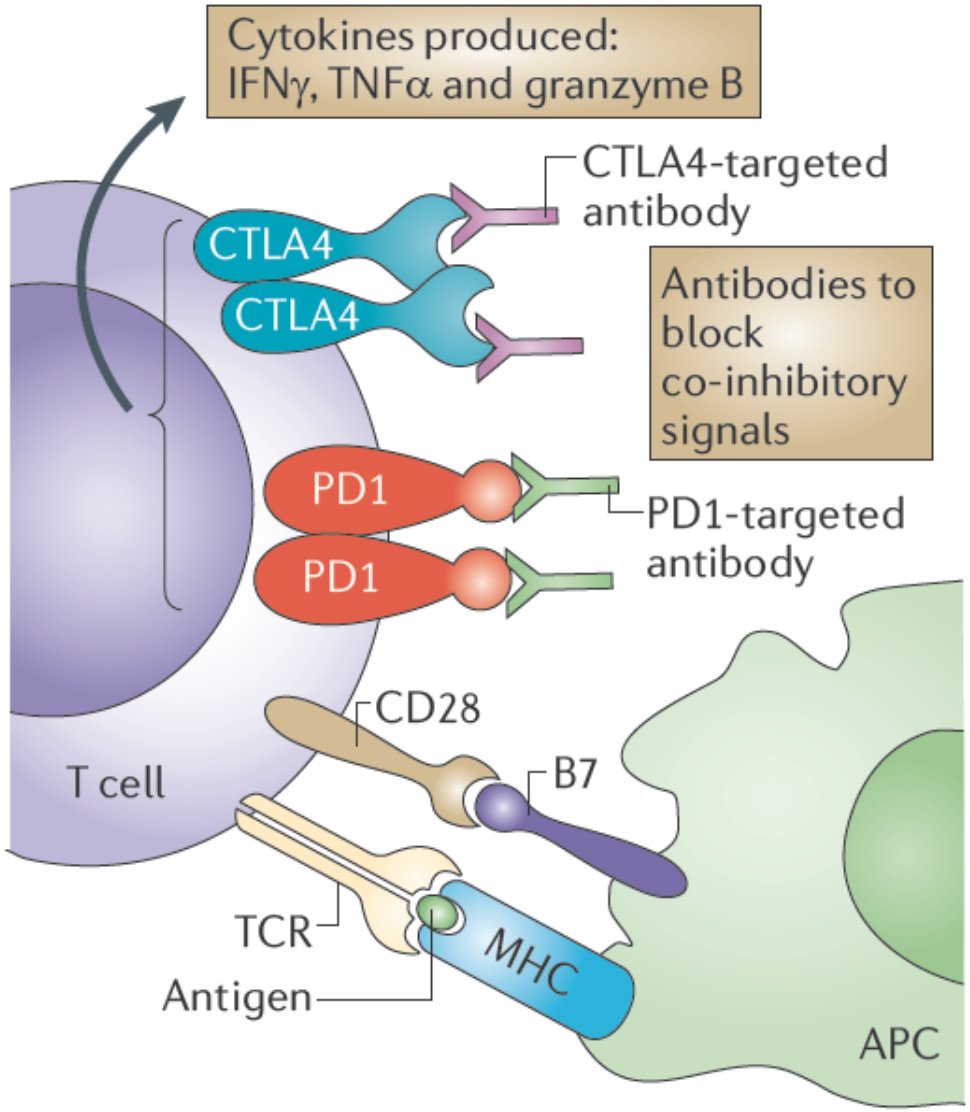
ALK vaccine combined with crizotinib delays lung tumor relapse





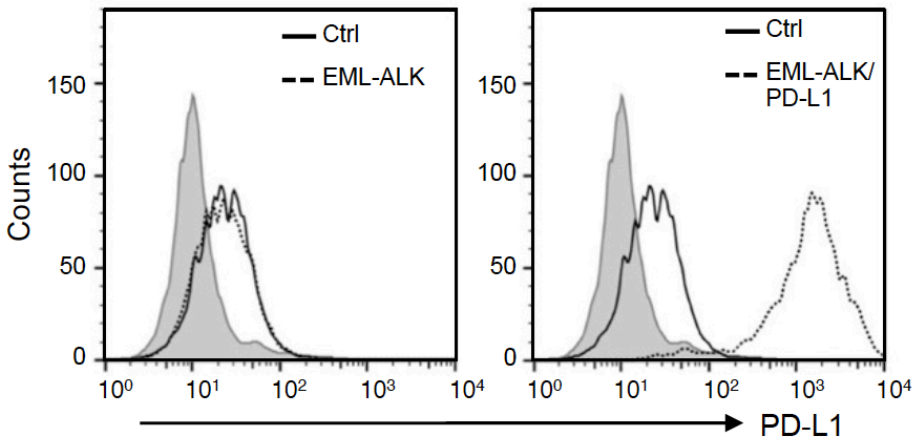


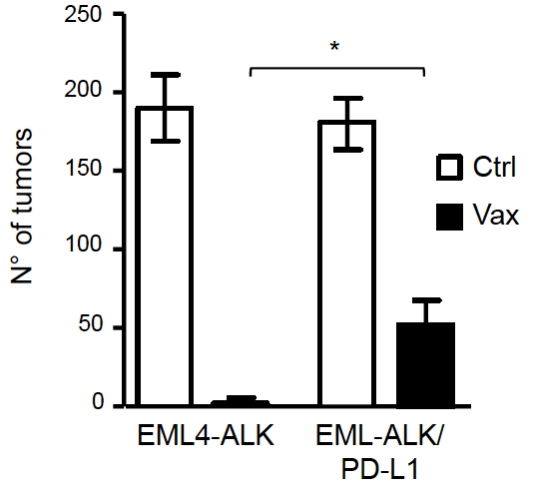
Immune checkpoint blockade in cancer therapy

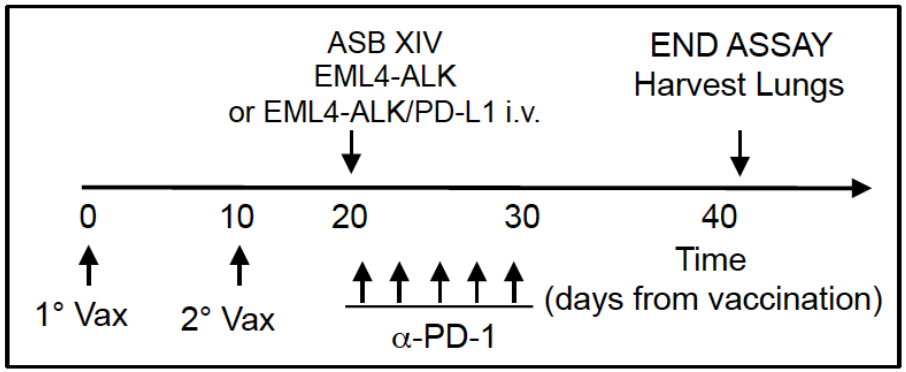


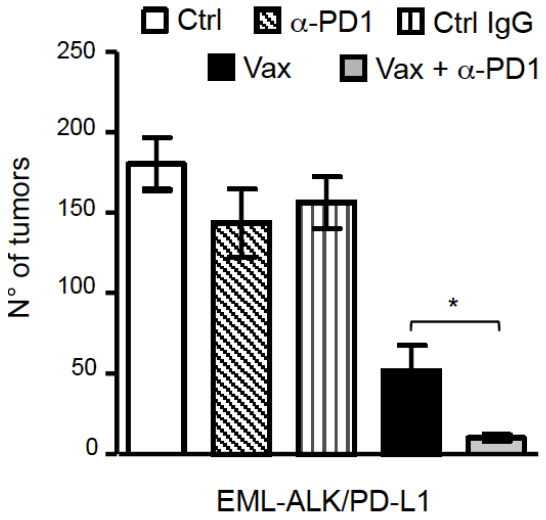
Sharma et al, Nat Rev Cancer 2011

Blockade of PD-1/PD-L1 enhances ALK vaccine efficacy









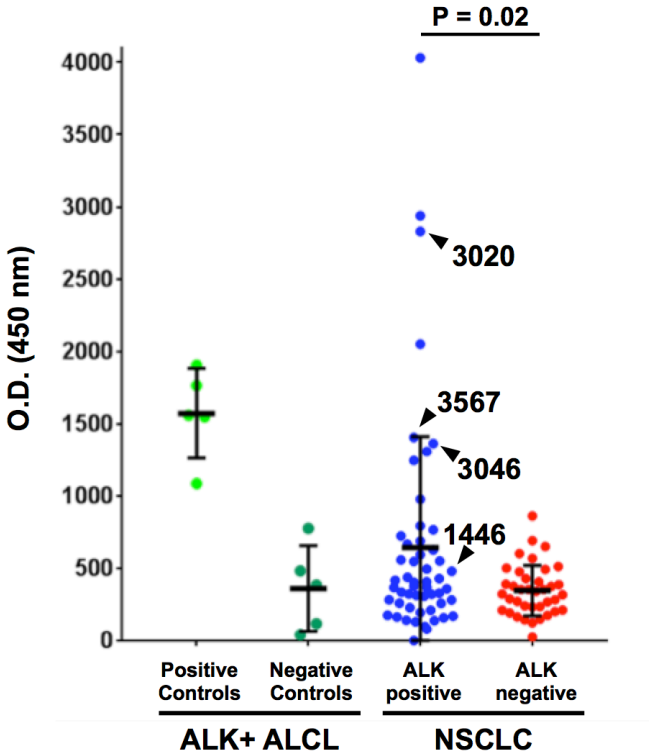
Voena et al, *Cancer Res Immunol* 2015

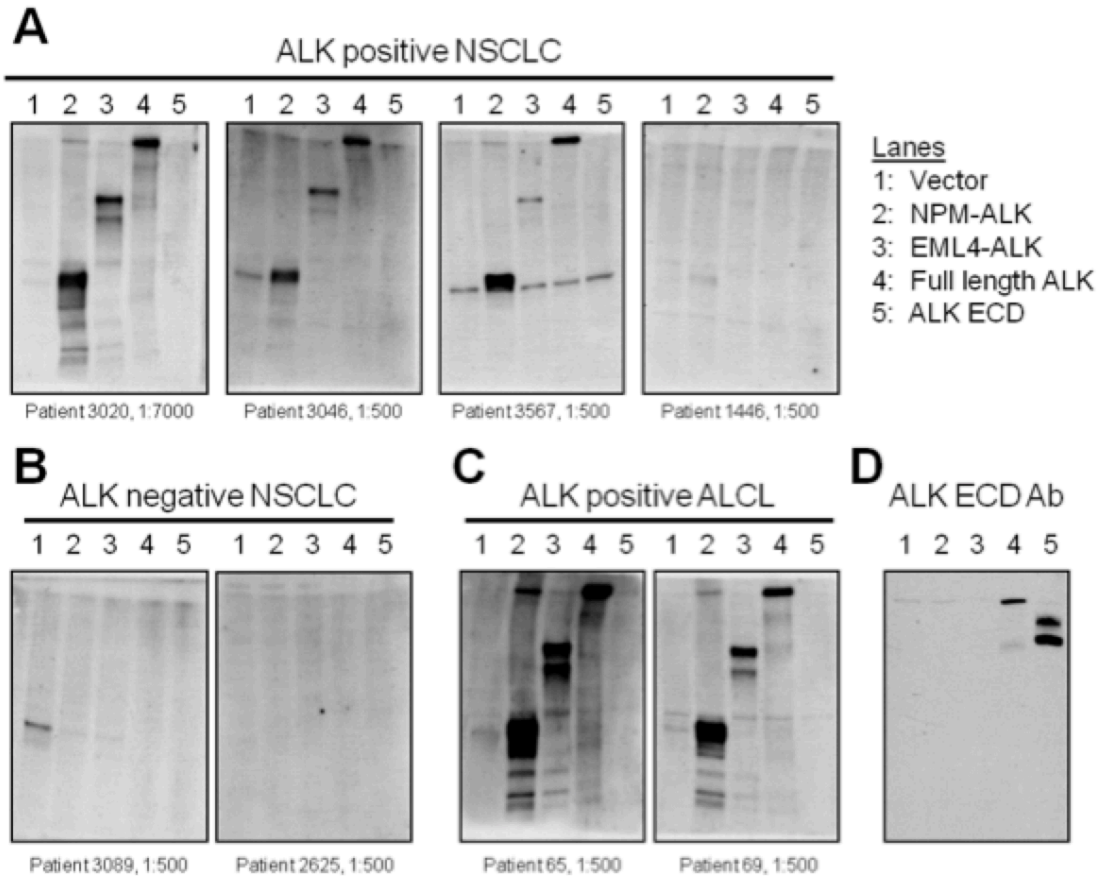
Conclusions from preclinical studies on ALK vaccine in NSCLC

- ALK vaccine elicits a strong cytotoxic immune response against ALK-rearranged NSCLC cells
- ALK vaccine reduces tumor growth and extends survival in NSCLC models
- ALK vaccine in combination with ALK inhibitors delays tumor relapse
- PD-L1 is regulated by EML4-ALK in NSCLC
- Immunocheckpoint blockade by PD-1/PD-L1 antibodies increases ALK vaccine efficacy
- ALK vaccine is active against crizotinib-resistant ALK mutants

Moving toward the clinical use of an ALK vaccine

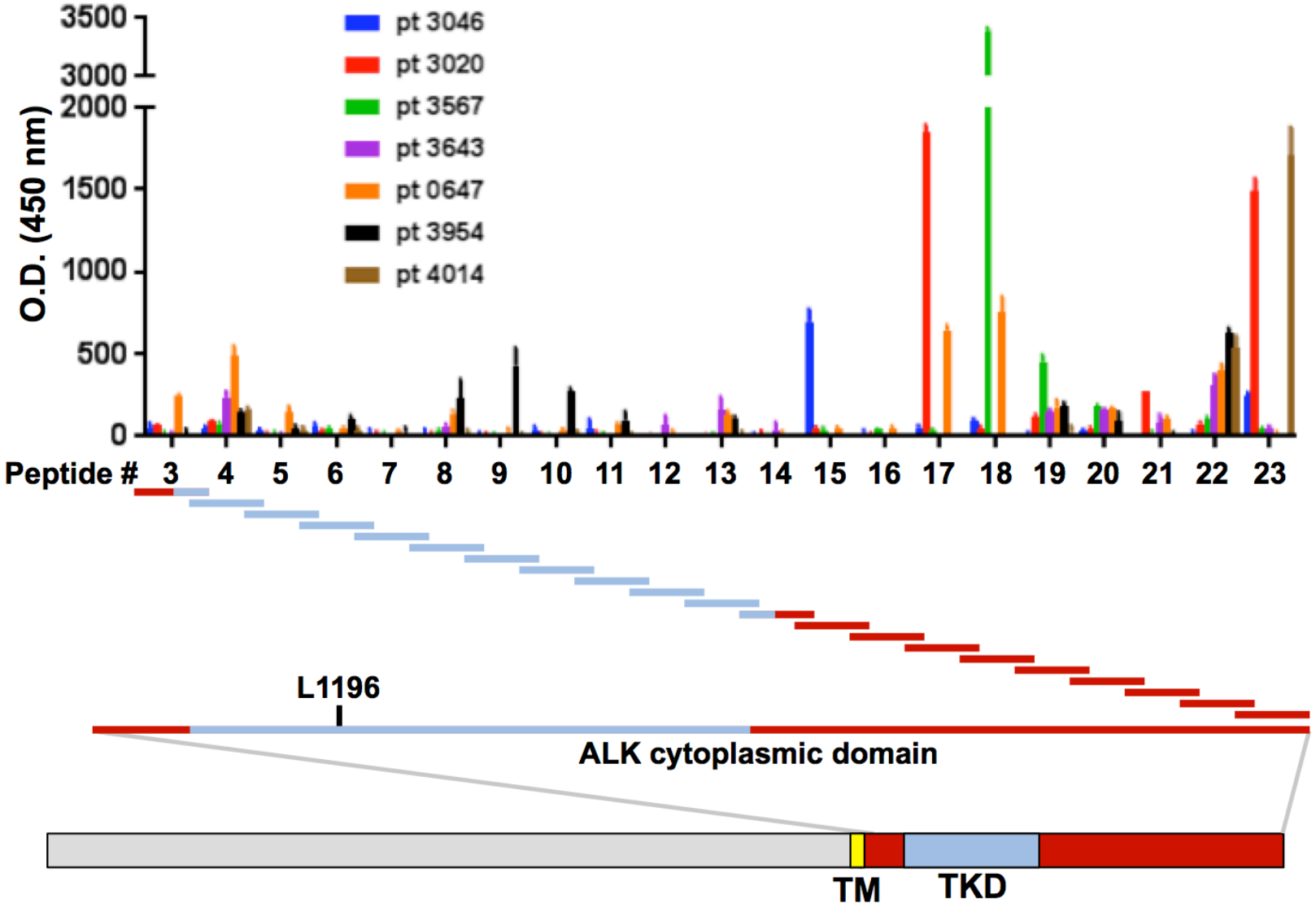
Anti-ALK immunity in NSCLC patients





In collaboration with Mark M. Awad (DFCI)

Anti-ALK immunity in NSCLC patients



Awad et al., Oncotarget 2017

Anti-ALK immunity in NSCLC patients

HLA A*0201

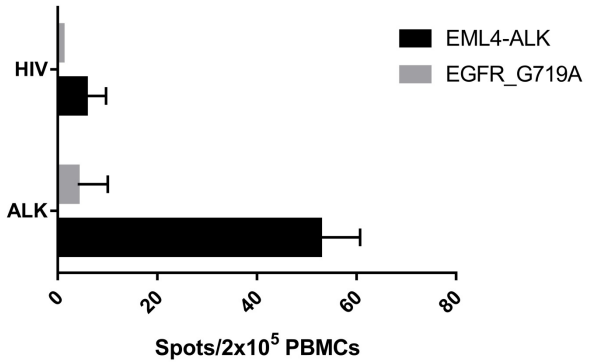
nMHC net		IEDB	
9mer	nM	9mer	Percentile Rank
VLLWEIFSL	5	VLLWEIFSL	0.3
AMLDLLHVA	8	FLMEALIIS	0.5
KTDTWSFGV	11	AMLDLLHVA	0.5
FLMEALIIS	16	KTDTWSFGV	1.1
GMARDIYRA	77	GMARDIYRA	1.2
10mer	nM	10mer	Percentile Rank
SLAMLDLLHV	16	SLAMLDLLHV	0.4
FLMEALIISK	35	FLMEALIISK	0.55
SLPRFILLEL	53	SLPRFILLEL	1.45
LLLEPSSLTA	71	GVLLWEIFSL	1.55
GVLLWEIFSL	93	LLLEPSSLTA	1.6

HLA B*0702

MHCnet		IEDB	
9mer	nM	9mer	Percentile Rank
KPTKKNNPI	14	RPSQPSSLA	0.3
RPSQPSSLA	16	LPRFILLEL	0.5
LPRFILLEL	18	VPRKNITLI	0.5
IVRCIGVSL	20	KPTKKNNPI	1.1
VPRKNITLI	27	RPRPSQPSS	1.2
10mer	nM	10mer	
RPRPSQPSSL	7	RPRPSQPSSL	0.1
RPSQPSSLAM	14	RPSQPSSLAM	0.25
YPSKSNQEVL	55	LPRFILLELM	0.3
LPRFILLELM	60	YPSKSNQEVL	0.65
SPLQVAVKTL	85	SPLQVAVKTL	0.85

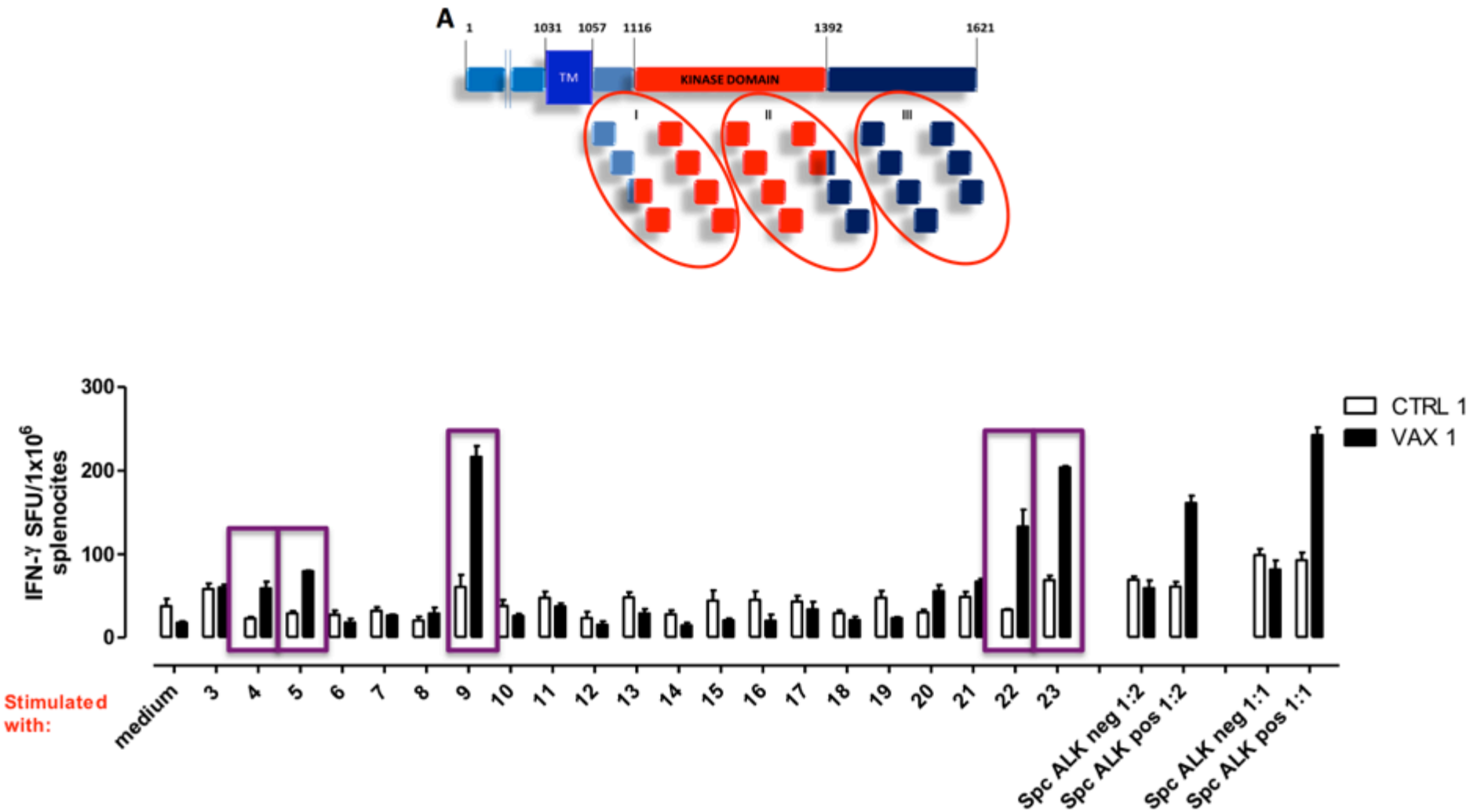
HLA C*0702

MHCnet		IEDB	
9mer	nM	9mer	Percentile.Rank
YYRKGGCAM	62	VYEGQVSGM	0.7
YRKGGCAML	194	YRKGGCAML	0.75
VYRRKHQEL	225	VYRRKHQEL	0.85
KWMPPEAFM	257	VRVPRGPAV	1.2
GRLPGASLL	316	YYRKGGCAM	1.35
10mer	nM	10mer	Percentile.Rank
YYRKGGCAML	64	YYRKGGCAML	0.2
SYYRKGGCAM	145	SYYRKGGCAM	0.5
ERSPAAPPPL	429	RRKHQELQAM	1.2
RRKHQELQAM	443	ERSPAAPPPL	1.2
GRLPGASLLL	495	SFGVLLWEIF	1.3

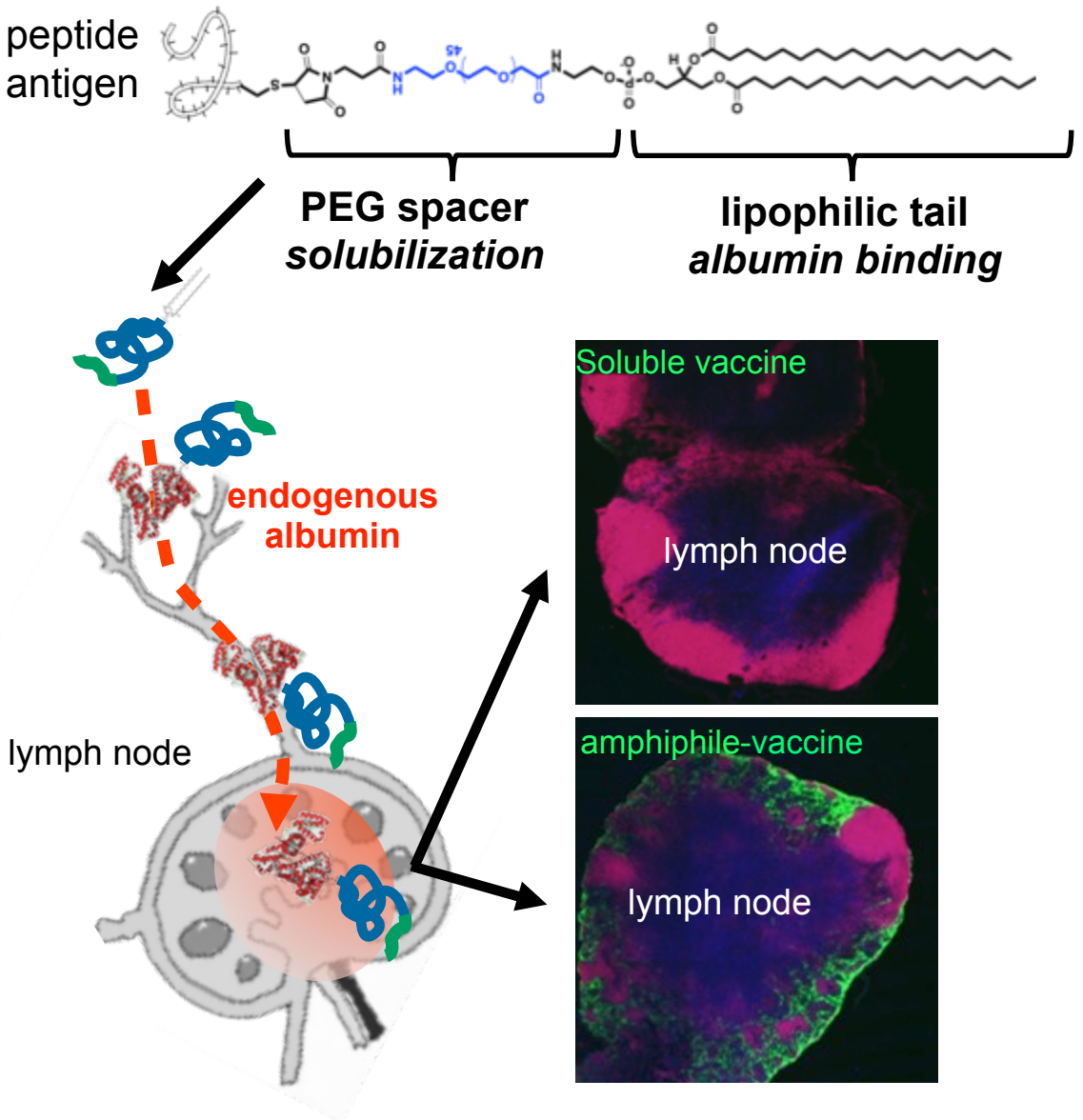


In collaboration with Mark M. Awad (DFCI)

Generation of a Synthetic Long Peptide ALK vaccine

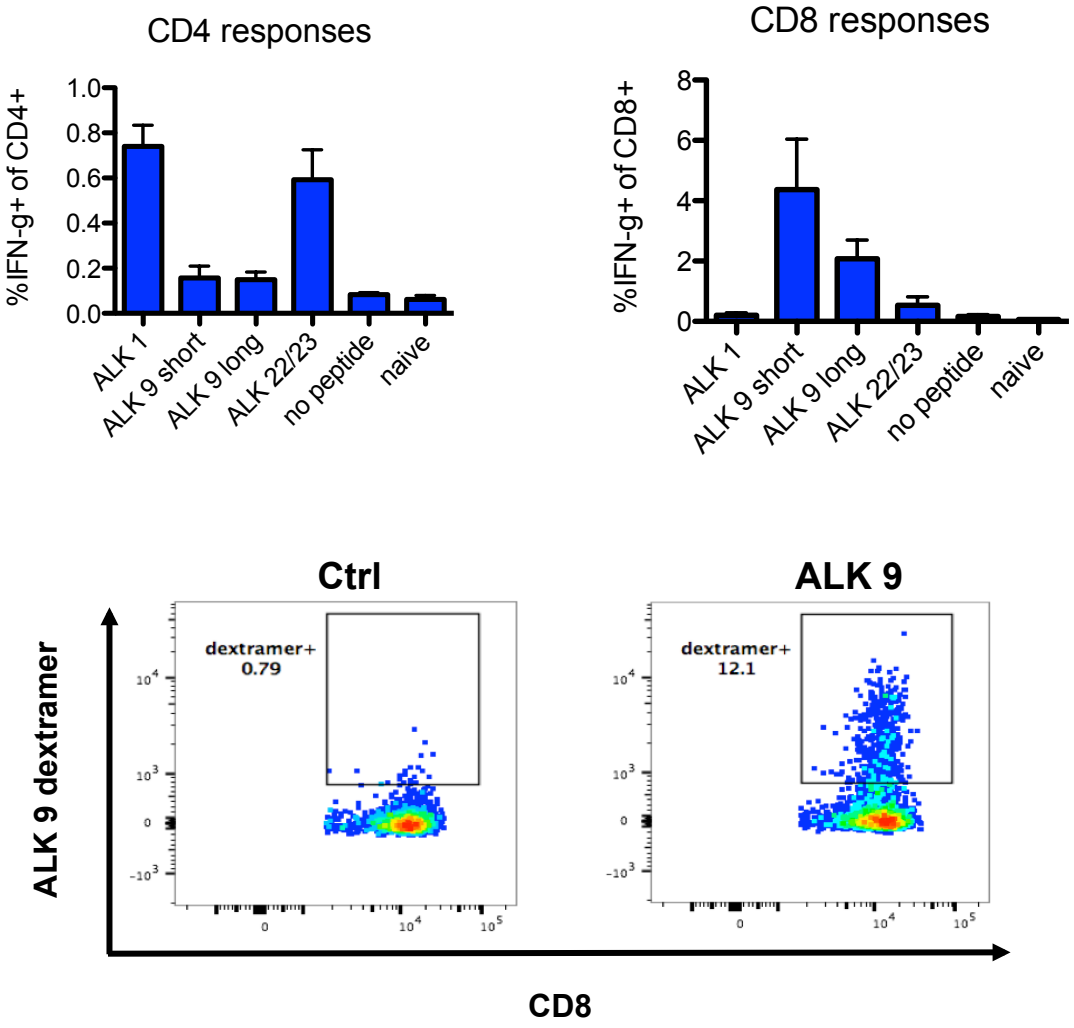


Lymph node targeting “albumin hitchhiking” vaccines



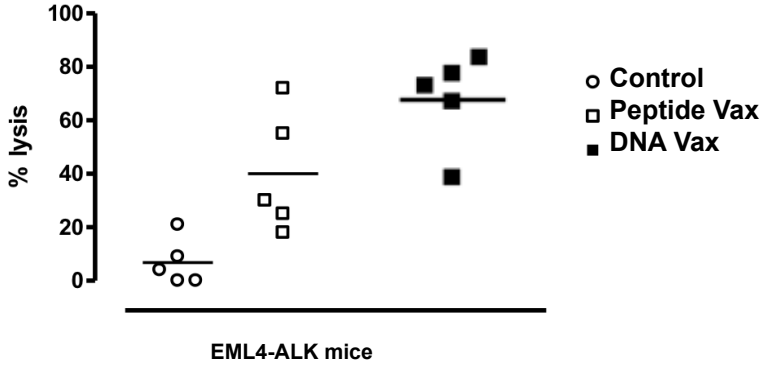
Liu et al. *Nature* 2014

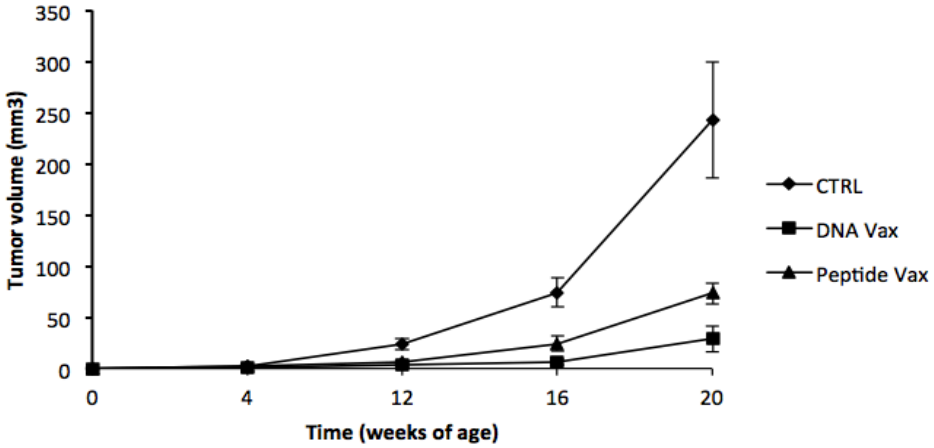
Efficacy of a peptide-based vaccine against ALK+ lung cancer



In collaboration with Darrell J. Irvine (MIT)

Efficacy of a peptide-based vaccine against ALK+ lung cancer



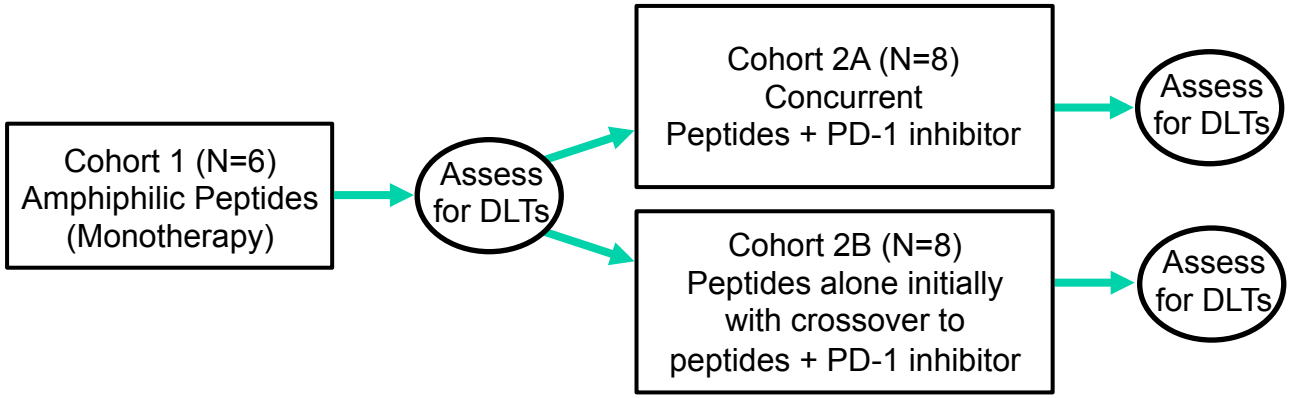


In collaboration with Darrell J. Irvine (MIT)

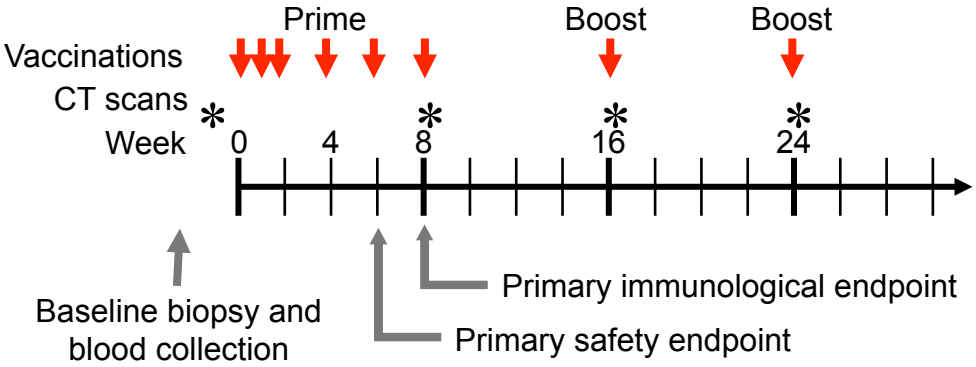
Phase I clinical trial to test efficacy of an ALK vaccine in NSCLC patients
In collaboration with DFCI and MIT

Dr. Mark M. Awad
Dr. Darrell J. Irvine

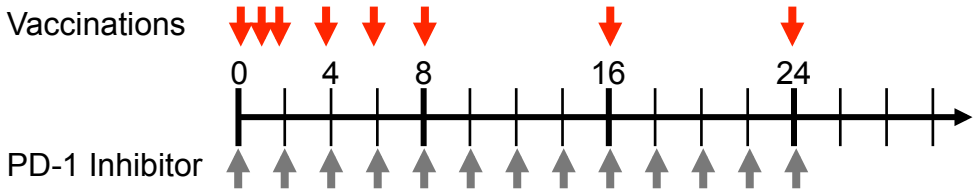
Phase 1 Trial: Schema



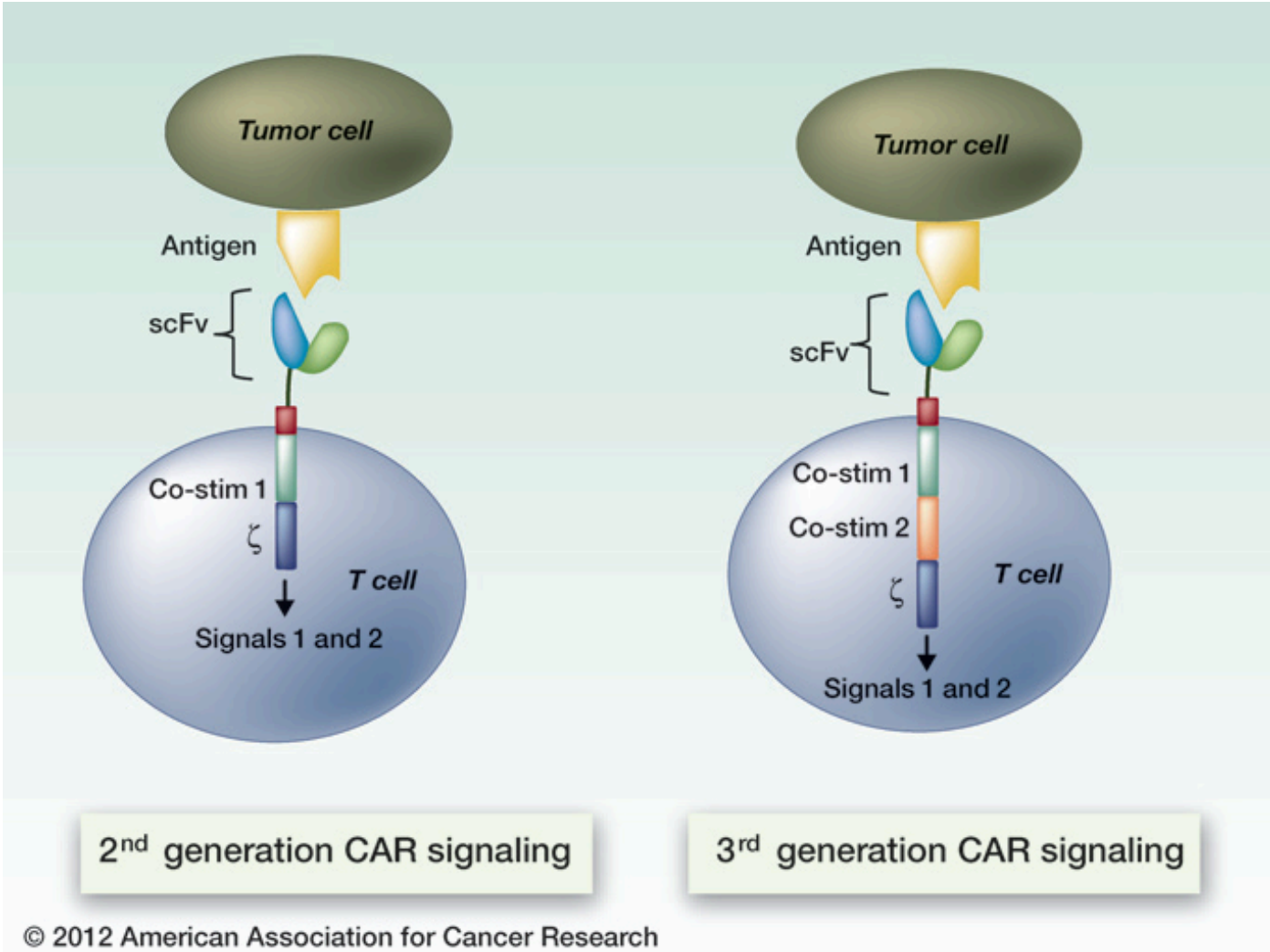
Cohort 1



Cohort 2A



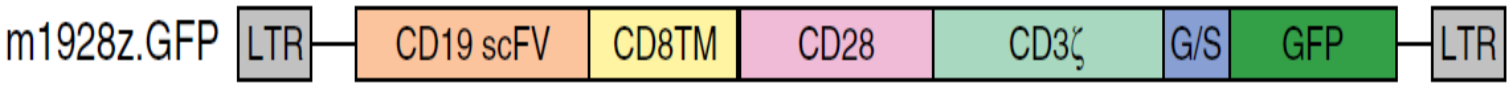
CAR T cell as novel tool for cancer immunotherapy



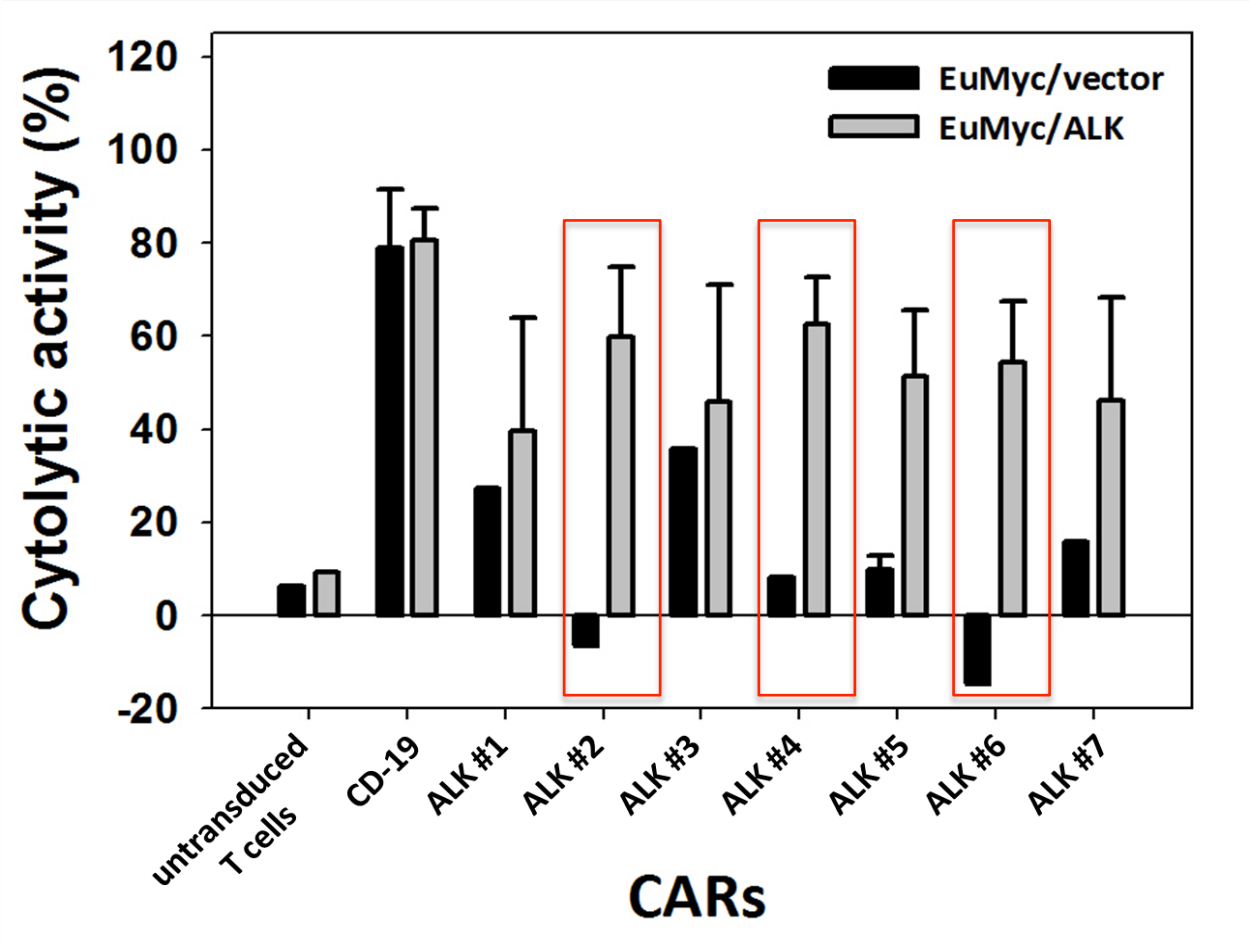
Antibodies used to generate ALK CAR T cell constructs.

ALK Ab	ALK phosphorylation	K_D^{app} [nM]	ALK turnover	Mouse-ALK binding	KTN#
1	No activity	0.35	no	moderate	118
2	Inhibitor	0.2	no	moderate	119
3	Weak inhibitor	0.5	no	no	120
4	strong agonist	0.5	strong	no	123
5	Inhibitor	0.5	no	strong	125
6	Inhibitor	0.4	strong	strong	130
7	Weak Agonist	0.5	strong	strong	131

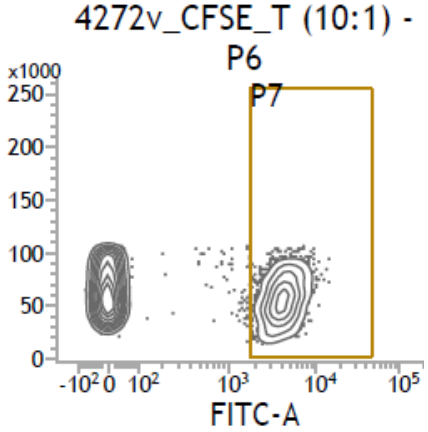
M1928z (CAR-CD19), provided by Dr. Michel Sadelain



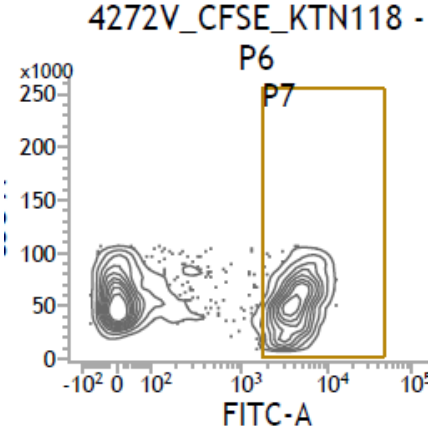
Development of ALK CAR T cells



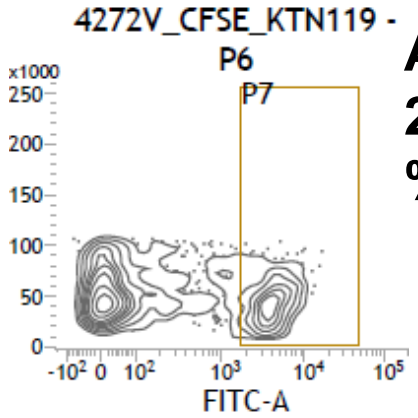
Eμ−myc4272-VECTOR



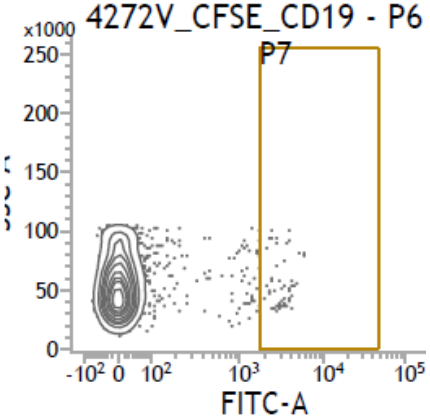
T
36.58
%



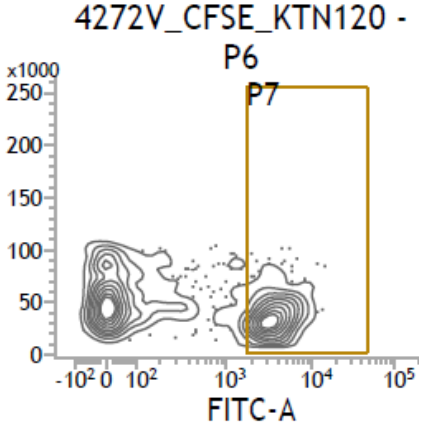
ALK#1
47.47
%



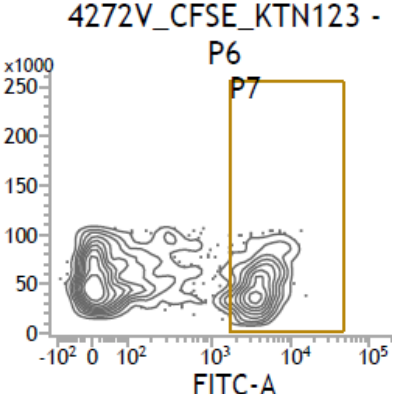
ALK#2
26.01
%



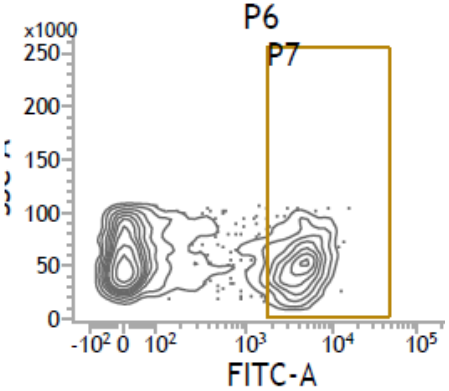
CD19
1.63%



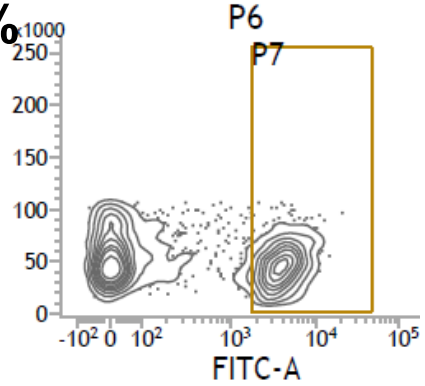
ALK#3
39.93
%



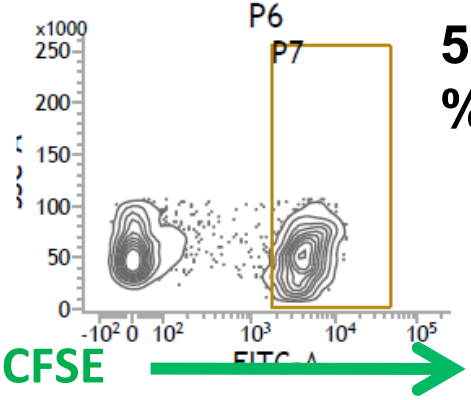
ALK#4
32.86
%



ALK#5
30.65%



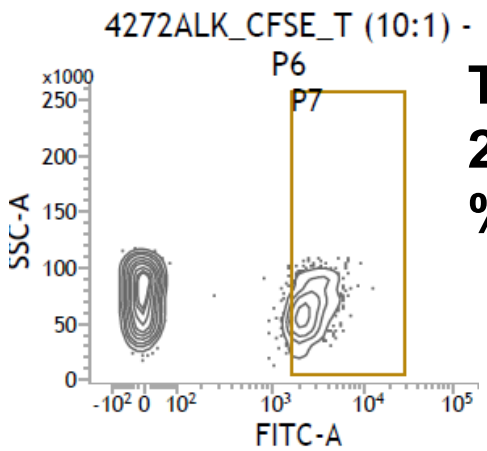
ALK#6
39.34
%



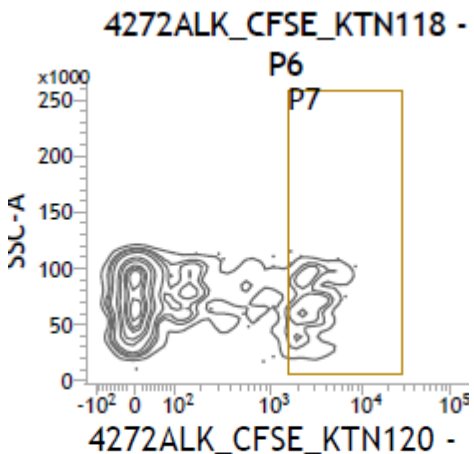
ALK#7
52.99
%

CFSE

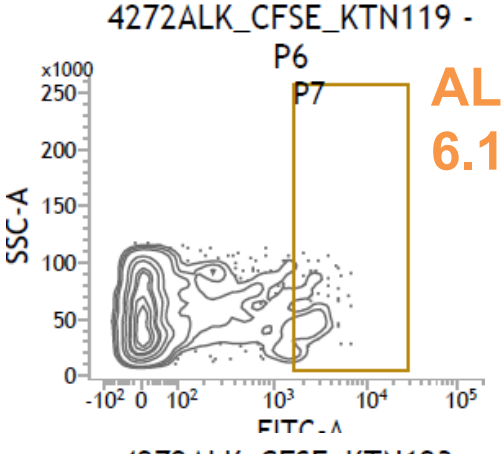
Eμ−myc4272-ALK



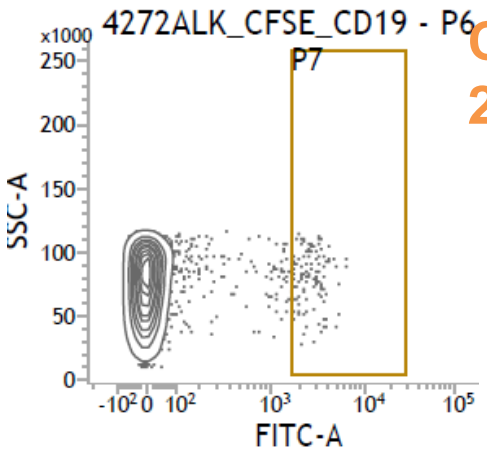
T 27.49 %



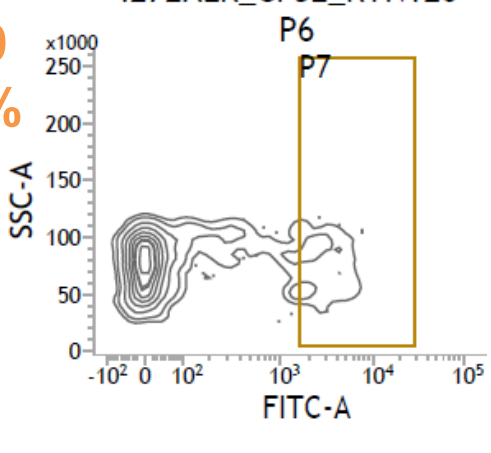
ALK#1 13.69 %



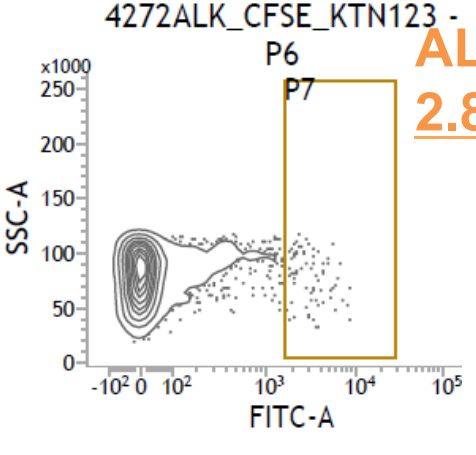
ALK#2 6.16%



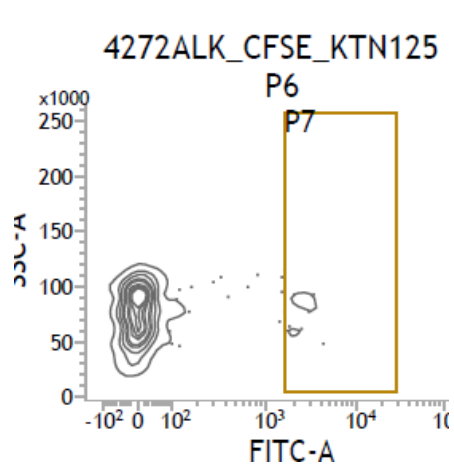
CD19 2.64%



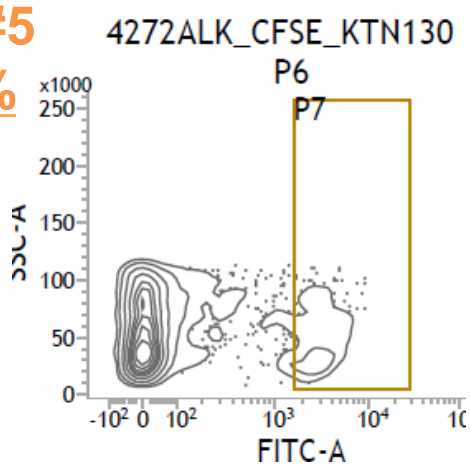
ALK#3 9.52%



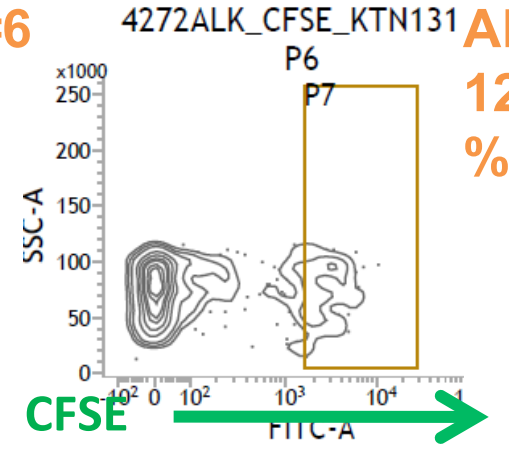
ALK#4 2.8%



ALK#5 3.41%

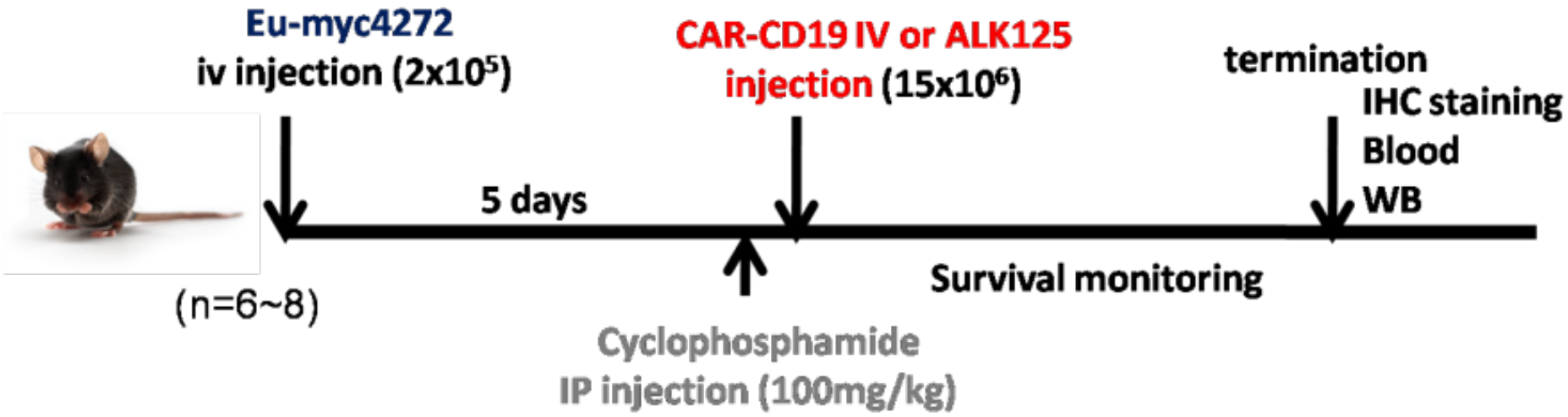


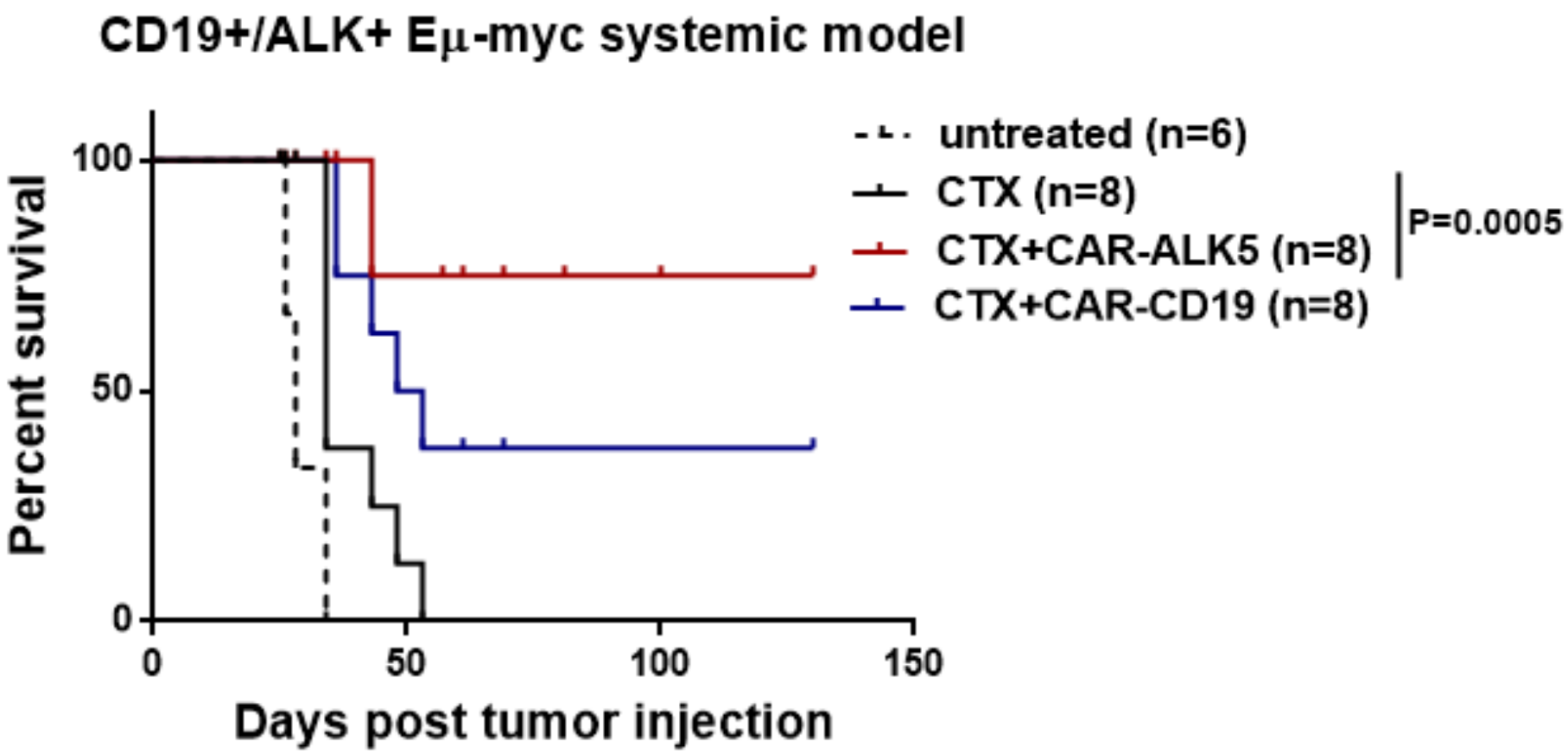
ALK#6 9.3%



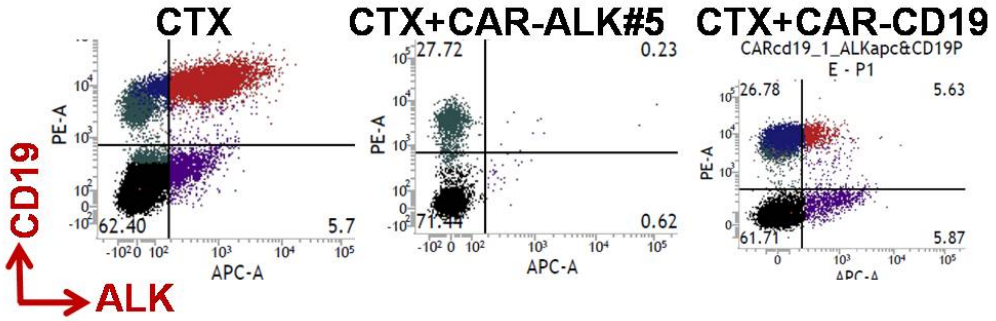
ALK#7 12.01 %

ALK-specific CAR #5 T cells showed a significant tumor-free survival in CD19+/ALK+ systemic C57BL/6J mice model.





CD19+/ALK+ circulating tumor cells in peripheral blood of mice.

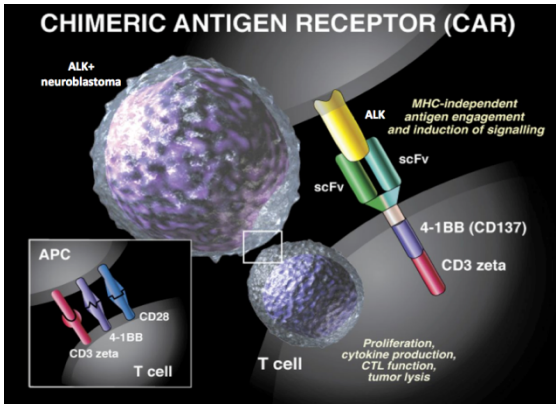


Therapeutic synergy between ALK vaccine and ALK CAR T cells

Debulk neuroblastoma - chemo/radiotherapy

Deliver ALK vaccine

Inject anti-ALK CAR T cells



First-of-its-kind combination therapy for neuroblastoma

Children's Hospital Boston
Department of Pathology

Harvard Medical School
Department of Pathology

Boston Children Hospital, Harvard Medical School

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Taek-Chin Cheong
Andy Wang
Wei-Tien Tai

David Williams
Hee Ho Park

Manuel Nuesch
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Claudia Voena
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Valerio Minero
Filomena Di Giacomo
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Federica Cavallo
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Mark M. Awad
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Mohit Butaney
Marzia Capelletti
Pasi A. Jänne

MIT

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NIH NATIONAL CANCER INSTITUTE

Translational Research Program
Boston Children's Hospital

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ALK inhibitors resistance mechanisms in NSCLC

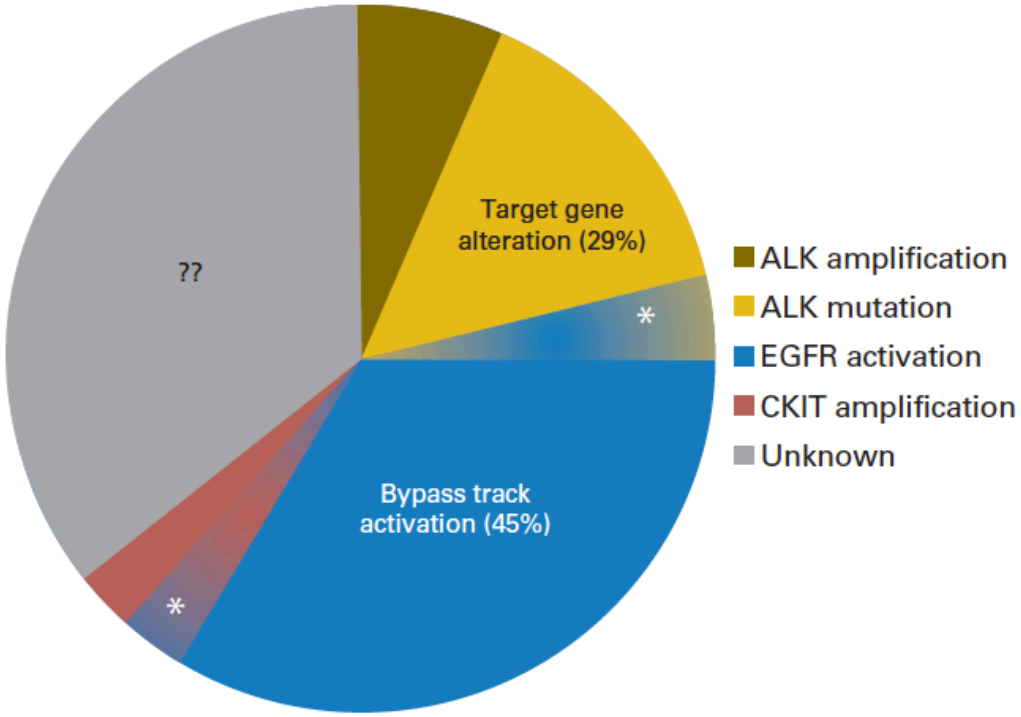
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

N ENGL J MED 368;25 NEJM.ORG JUNE 20, 2013

Crizotinib versus Chemotherapy in Advanced *ALK*-Positive Lung Cancer

Alice T. Shaw, M.D., Ph.D., Dong-Wan Kim, M.D., Ph.D., Kazuhiko Nakagawa, M.D., Ph.D., Takashi Seto, M.D., Lucio Crinó, M.D., Myung-Ju Ahn, M.D., Tommaso De Pas, M.D., Benjamin Besse, M.D., Ph.D., Benjamin J. Solomon, M.B., B.S., Ph.D., Fiona Blackhall, M.D., Ph.D., Yi-Long Wu, M.D., Michael Thomas, M.D., Kenneth J. O'Byrne, M.D., Denis Moro-Sibilot, M.D., D. Ross Camidge, M.D., Ph.D., Tony Mok, M.D., Vera Hirsh, M.D., Gregory J. Riely, M.D., Ph.D., Shrividya Iyer, Ph.D., Vanessa Tassell, B.S., Anna Polli, B.S., Keith D. Wilner, Ph.D., and Pasi A. Jänne, M.D., Ph.D.



Rodig et al, Clin Cancer Res 2009
Shaw and Engelman, JCO, 2013

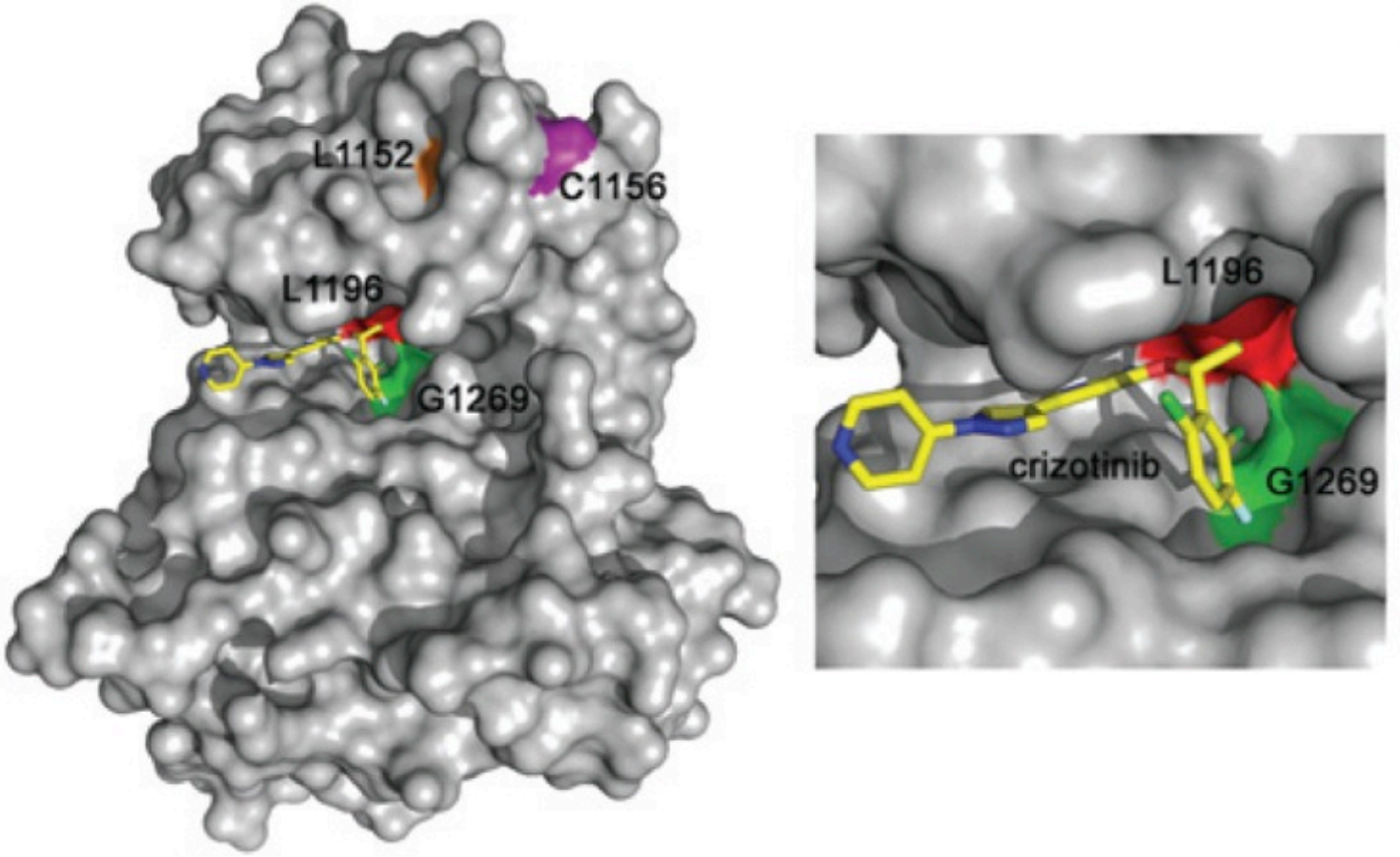
ALK mutations that confer resistance to crizotinib

Mutation	ALK Fusion	Sensitivity to ALK inhibitors	Disease	Reference
L1152R	EML4-ALK E6:A20	ND	NSCLC	*Sasaki T, et al. 2011*
C1156Y	EML4-ALK E13:A20	ND	NSCLC	*Choi YL, et al. 2010* *Katayama R, et al. 2011*
F1174L	RANBP2-ALK	High dosage crizotinib	IMT Neuroblastoma	*Bresler SC, et al. 2011* *Sasaki T, et al. 2010*
L1196M	EML4-ALK E13:A20	AP26113, CH5424802	NSCLC	*Katayama R, et al. 2011* *Sakamoto H, et al. 2011*
G1202R	ND	CH5424802, ASP3026	NSCLC	*Katayama R, et al. 2012*
S1206Y	ND	CH5424802, ASP3026	NSCLC	*Katayama R, et al. 2012*
G1269A	EML4-ALK	ND	NSCLC	*Doebele, et al. 2012*
F1174C	EML4-ALK	ND	NSCLC	*Doebele, et al. 2012*
D1203N	EML4-ALK	ND	NSCLC	*Doebele, et al. 2012*
1151Tins	ND	CH5424802	NSCLC	*Katayama R, et al. 2012*
L1196Q	NPM-ALK	AP26113, NVP-TAE684	ALCL	*Ceccon, et al. 2013*
I1171N	NPM-ALK	High dosage crizotinib	ALCL	*Ceccon, et al. 2013*

Plasma concentration of crizotinib at the recommended dose (250mg twice daily) is 0.57 mmol/L

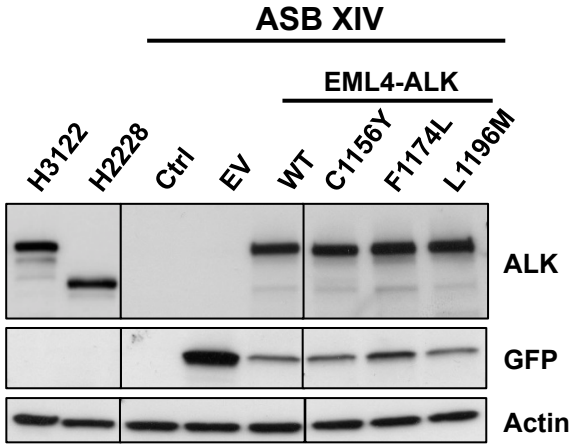
The ALK-L1196M mutation is equivalent to the gatekeeper mutations epidermal growth factor receptor (EGFR)-T790M

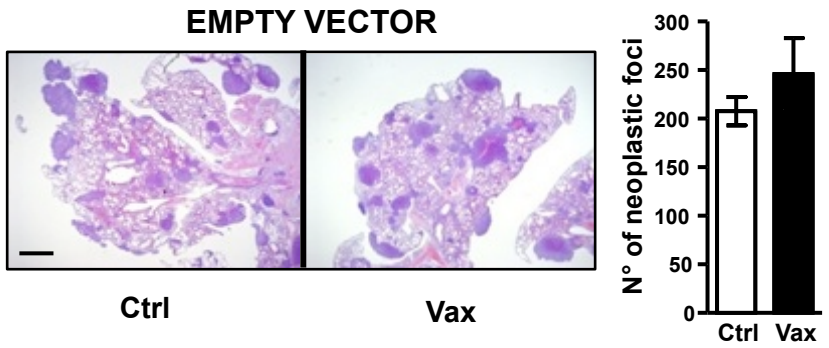
ALK mutations that confer resistance to crizotinib

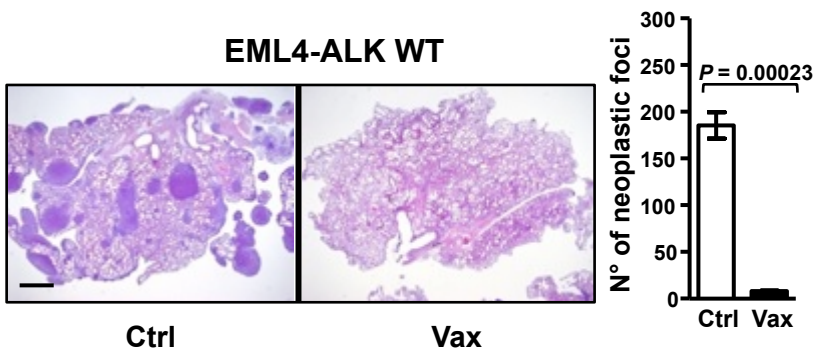


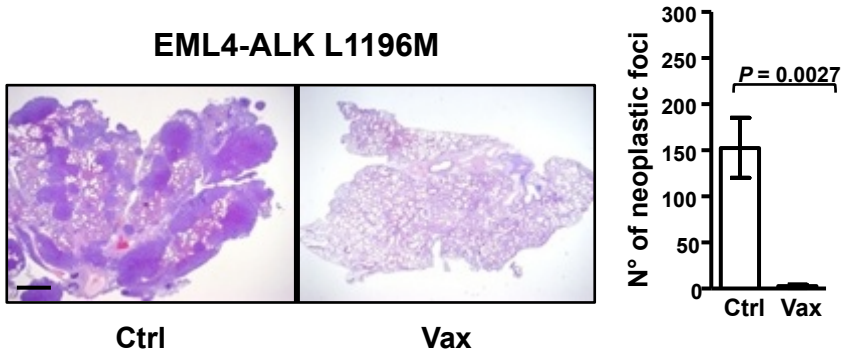
Doebele et al. Clin Cancer Res, 2012
Katayama et al., Science Trans Med, 2012

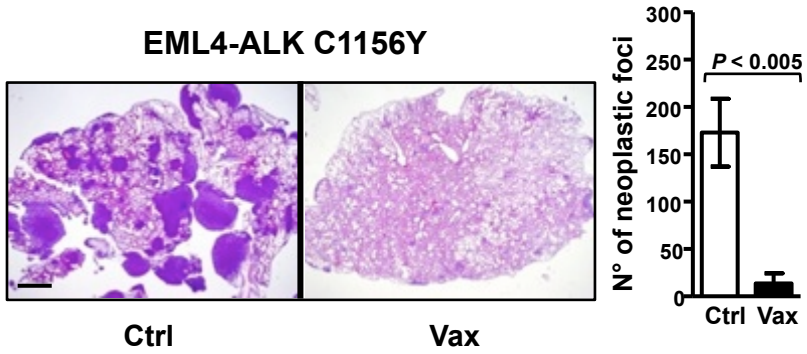
ALK vaccine targets crizotinib-resistant ALK mutations

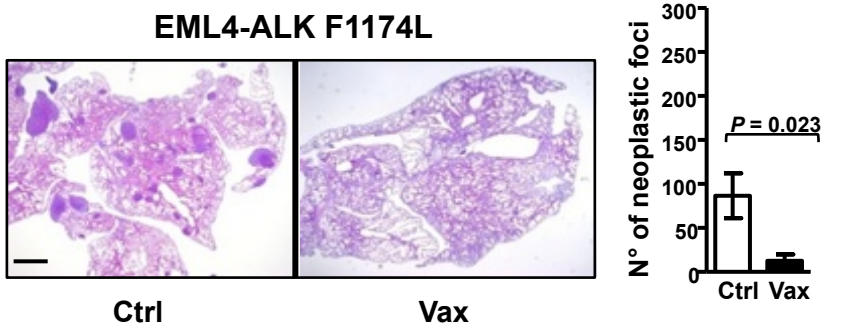






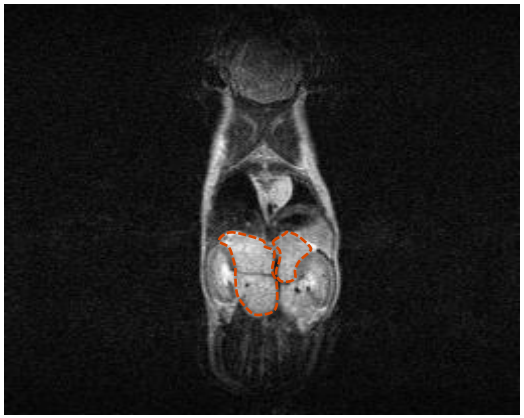


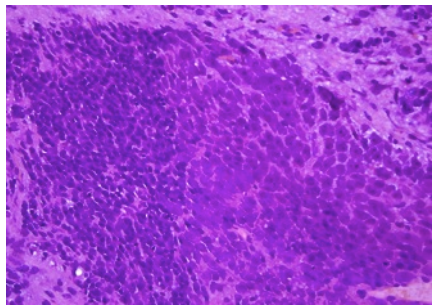




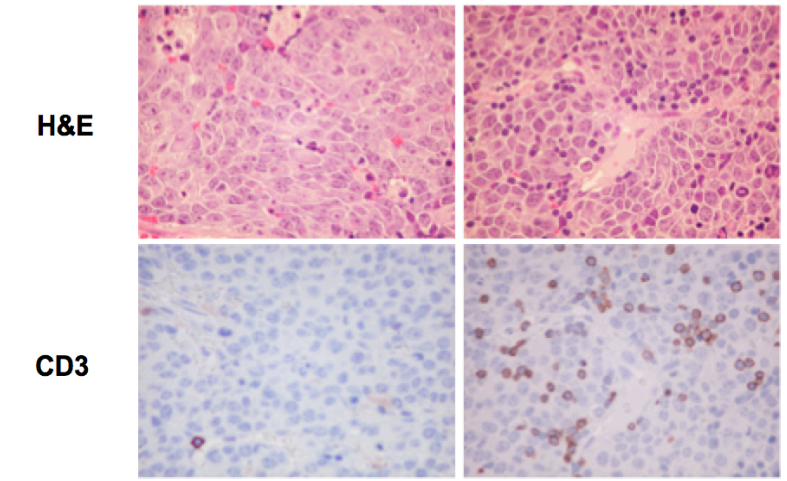
ALK vaccine in neuroblastoma

ALK^{F1174L}/MYCN





A



B