3RD ANNUAL COURSE OF PHARMACOGENETICS AND PERSONALIZED MEDICINE

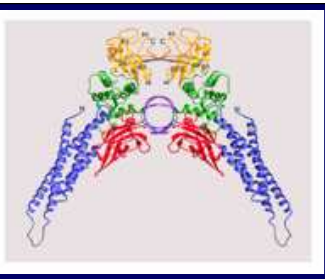
Emerging pathways in Personalized Medicine: breaking barriers and moving forward

STAT3: a pivotal target for individualized treatment of cancer

Thursday 9th - Friday 10th
February 2012

GIORGIO INGHIRAMI
Department of Biomedical Science and Human Oncology
Center of Experimental Medicine and Research (CeRMS) University of Turin

CeRMS
Centro di Ricerca in Medicina Sperimentale



THE JAK-STAT PATHWAY: 20 YEARS FROM DISCOVERY TO DRUGS

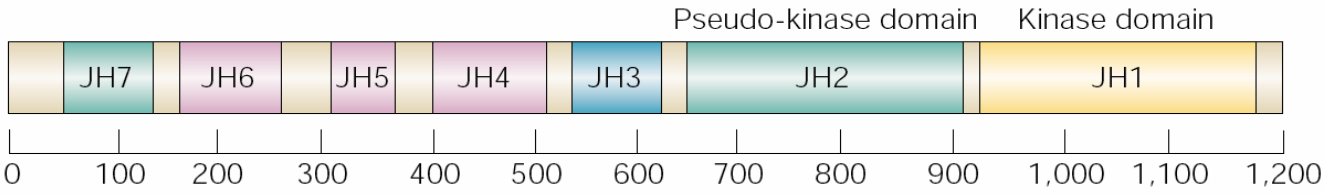
SEPTEMBER 22-24, 2011

National Institutes of Health, Bethesda, MD

Janus Kinase (JAK) family of tyrosine kinases

Family members

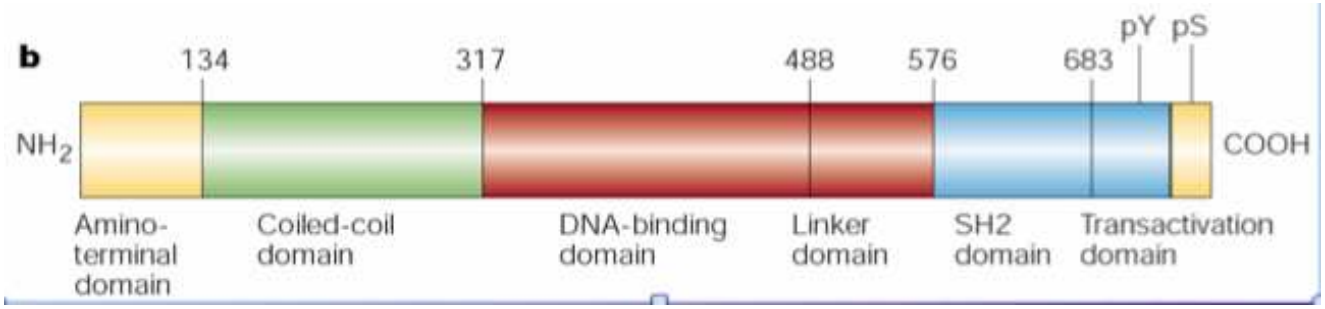
- JAK1
- JAK2
- JAK3
- Tyk2



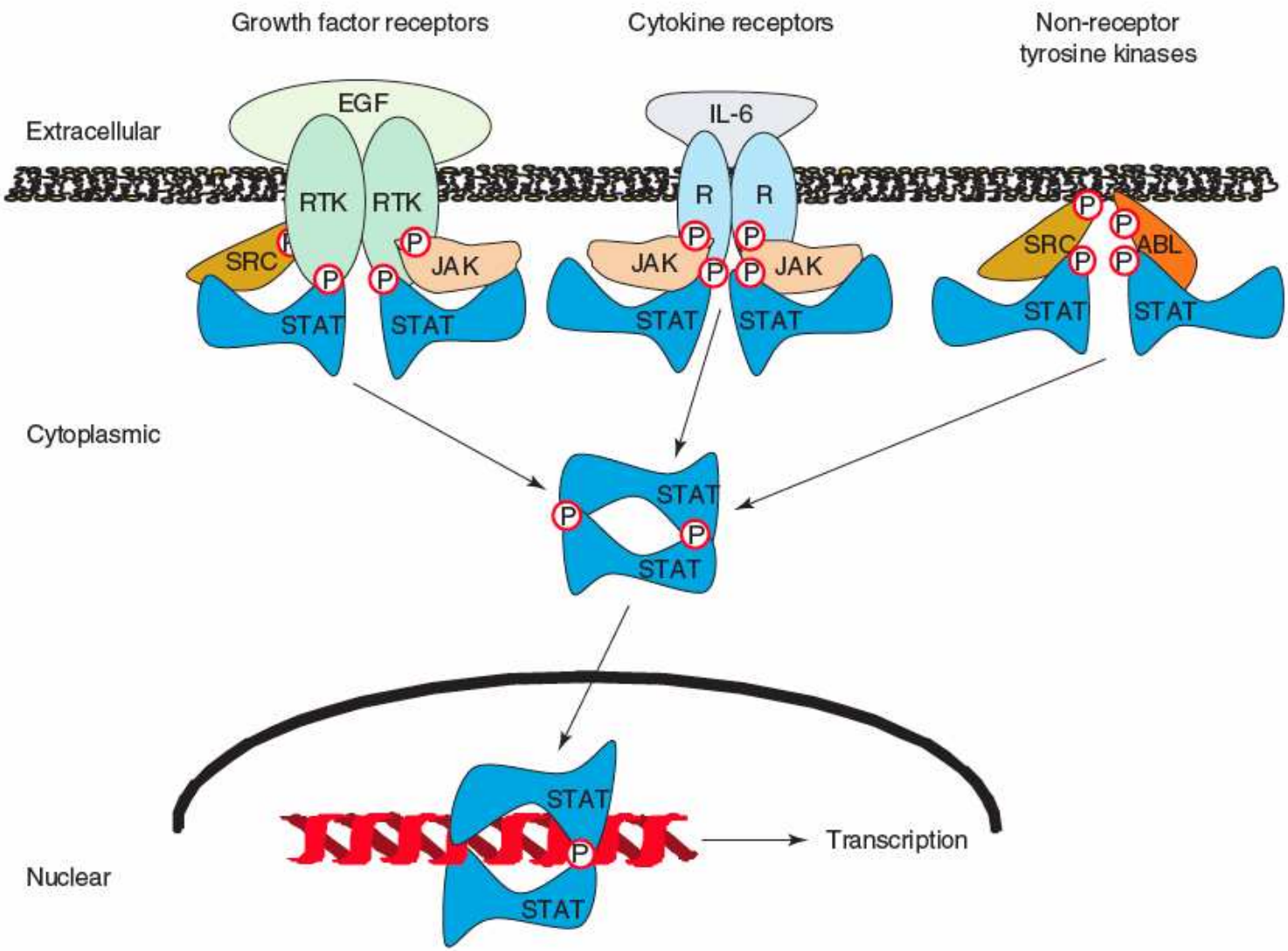
STATs: Signal Transducers and Activators of Transcription

Family members

STAT1	STAT2
STAT3	STAT4
STAT5A/B	STAT6



Canonical STAT3 signaling



Non Canonical STAT3 signaling

STAT3: A multifaceted oncogene

David E. Levy*† and Giorgio Inghirami*‡

*Departments of Pathology and Microbiology and NYU Cancer Institute, New York University School of Medicine, New York, NY 10016; and ‡Department of Biomedical Sciences and Human Oncology, University of Turin and Center for Experimental Research and Medical Studies, 10060 Turin, Italy

Signal transducers and activators of transcription (STAT) proteins are a family of transcription factors first characterized for their role in cytokine signaling. These versa-

surveillance by conferring properties of a T lymphocyte regulatory phenotype on a T cell lymphoma.

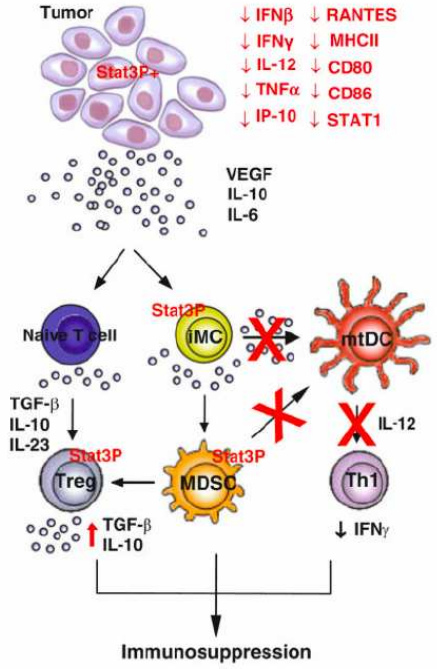
Stat3 and Immune Suppression

Kasprzycka *et al.* (5) show that ALK+ tumor cell lines secrete the inhibitory cytokines IL-10 and TGF-β, express CD25 and FoxP3, and condition their growth medium to be immunosuppres-

Stat3 regulates microtubules by antagonizing the depolymerization activity of stathmin

Dominic Chi Hiung Ng,[1] Bao Hong Lin,[1] Cheh Peng Lim,[1] Guochang Huang,[1] Tong Zhang,[1] Valeria Poli,[2] and Xinmin Cao[1]

[1]Signal Transduction Laboratory, Institute of Molecular and Cell Biology, Singapore 138673, Republic of Singapore
[2]Department of Genetics, Biology, and Biochemistry, University of Turin, 10126 Turin, Italy



Lee H et al Curr Top Microbiol Immunol.344: 4 (2011

The deregulated STAT3 activation is common event in human t

Table 1. STAT3 in the Context of Various Cancers: Validation as an Anticancer Target

Cancers Characterized by Elevated STAT3 Expression or Activity	Poor Prognosis Linked to High STAT3 Levels	Upstream/Downstream Abnormalities of STAT3 Signaling	Xenograft Models Responsive to Inhibition of STAT3
Leukemia	Renal cell carcinoma	Elevated EGFR expression	Head and neck squamous cell carcinoma
Lymphomas	Colorectal cancer	Constitutively activated EGFR-RTK	Glioblastoma
Multiple myeloma	Ovarian carcinoma	Overexpression of SFKs	Myeloproliferative neoplasms
Breast cancer	Gastric carcinoma	Hyperactivated JAKs	Renal cell carcinoma
Prostate carcinoma	Intestinal-type gastric adenocarcinoma	Elevated TGFα/IL-6	Breast cancer
Lung cancer (non-small-cell)	Cervical squamous-cell carcinoma		Lung adenocarcinoma
Renal cell carcinoma lung cancer	Osteosarcoma		Acute lymphoblastic leukemia
Hepatocellular carcinoma	Epithelial ovarian carcinoma		
Cholangiocarcinoma			
Ovarian carcinoma			
Pancreatic adenocarcinoma			
Melanoma			
Head and neck squamous cell carcinoma			

Johnston P.A. et al. Molecular Intervention 11, 18 (201

Peripheral T-cell lymphoma

Peripheral T-cell lymphoma (PTCL) are rare cancers, which account for about 12% of all NHL worldwide. They are a heterogeneous group of neoplasms that display great variability in their clinical, morphological, immunophenotypic, cytogenetic and molecular features. The PTCL can be roughly subdivided into: specified and not otherwise specified (NOS). While

Peripheral T cell lymphoma, not otherwise speci NOS)

Peripheral T cell lymphoma, specified

Leukaemic:

- T cell prolymphocytic leukaemia
- T cell large granular lymphocytic leukaemia
- Aggressive NK cell leukaemia
- Systemic Epstein–Barr virus positive T cell lympho disease of childhood (associated with chronic act infection)
- Hydroa vaccineforme-like lymphoma
- Adult T cell leukaemia/lymphoma

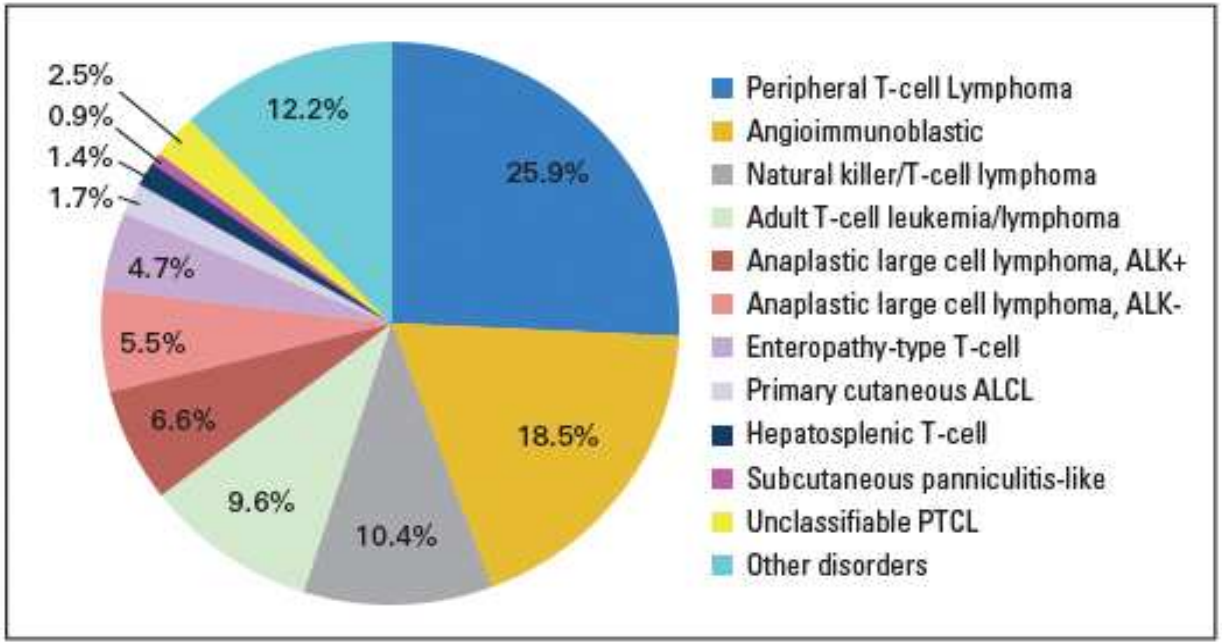
Extranodal:

- Extranodal NK/T cell lymphoma, nasal type
- Enteropathy-associated T cell lymphoma
- Hepatosplenic T cell lymphoma
- Subcutaneous panniculitis-like T cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous anaplastic large-cell lymphoma
- Primary cutaneous aggressive epidermotropic CD8 T cell lymphoma (provisional entity)
- Primary cutaneous γδ T cell lymphoma
- Primary cutaneous small/medium CD4+ T cell lymphoma (provisional entity)

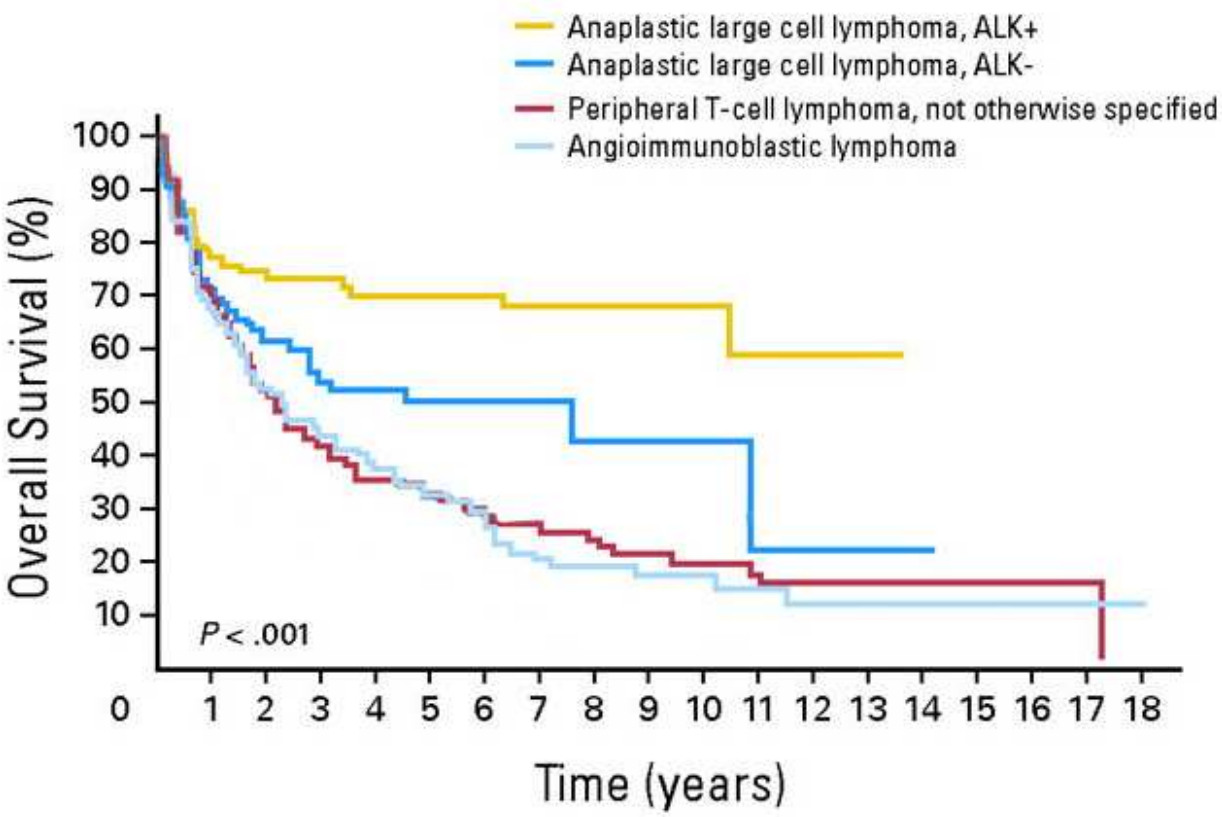
Prevalently nodal:

- Angioimmunoblastic T cell lymphoma
- Anaplastic large cell lymphoma (ALCL), anaplastic large cell lymphoma kinase (ALK) positive
- ALCL, ALK negative (provisional entity)

Overall incidence in USA ~5-6,000 cases a y



Overall Survival of patients with common Peripheral T-cell Lymphoma subtypes

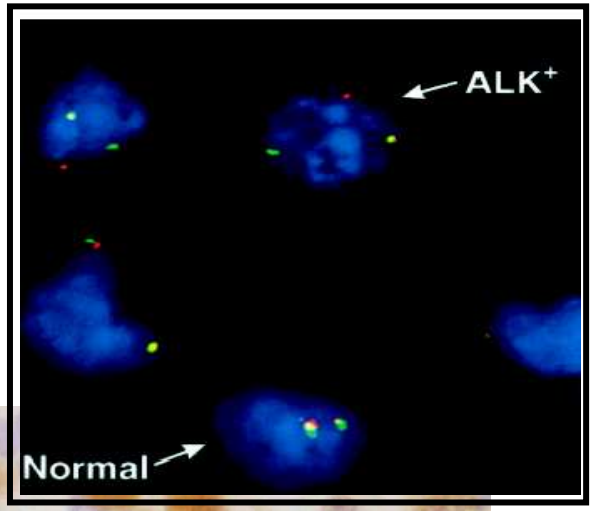


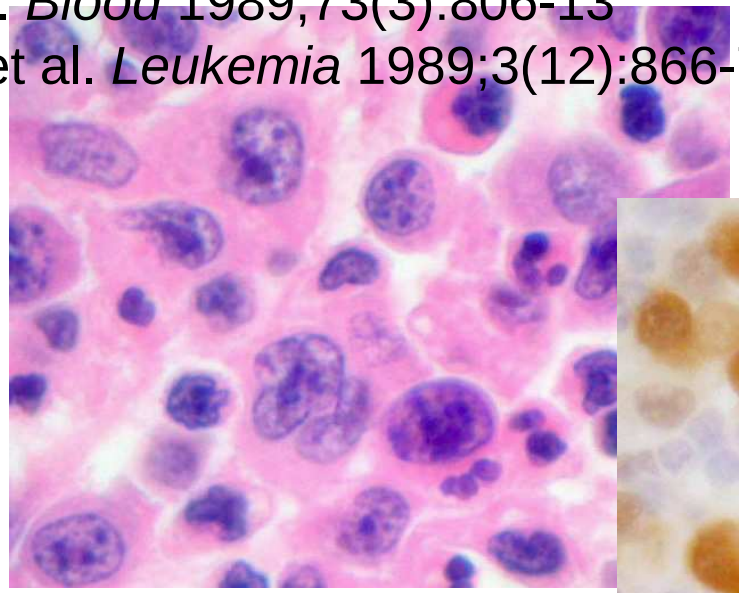
(Modified from Armitage et al. JCO 2008)

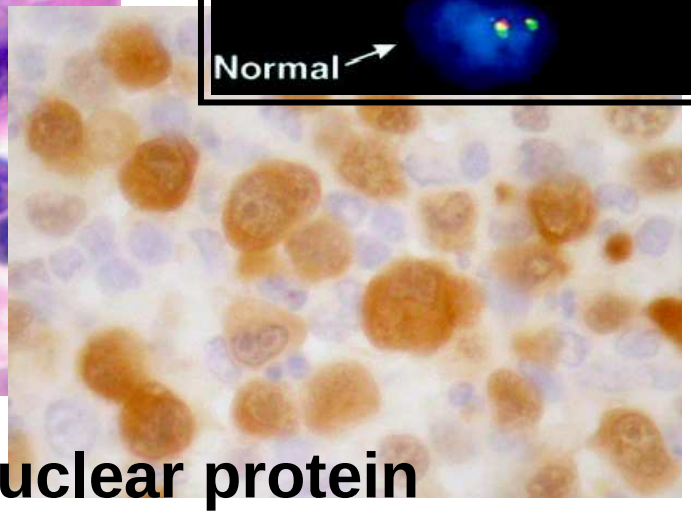
Anaplastic Large Cell Lymphoma

The t(2;5)(p23;q35) translocation is associated with Ki-1 (CD30+) lymphoma

Rimokh R. et al. *Br. J. Haematol* 1989;71(1):31-6
Keneko Y. et al. *Blood* 1989;73(3):806-13
Le Beau M.M. et al. *Leukemia* 1989;3(12):866-70

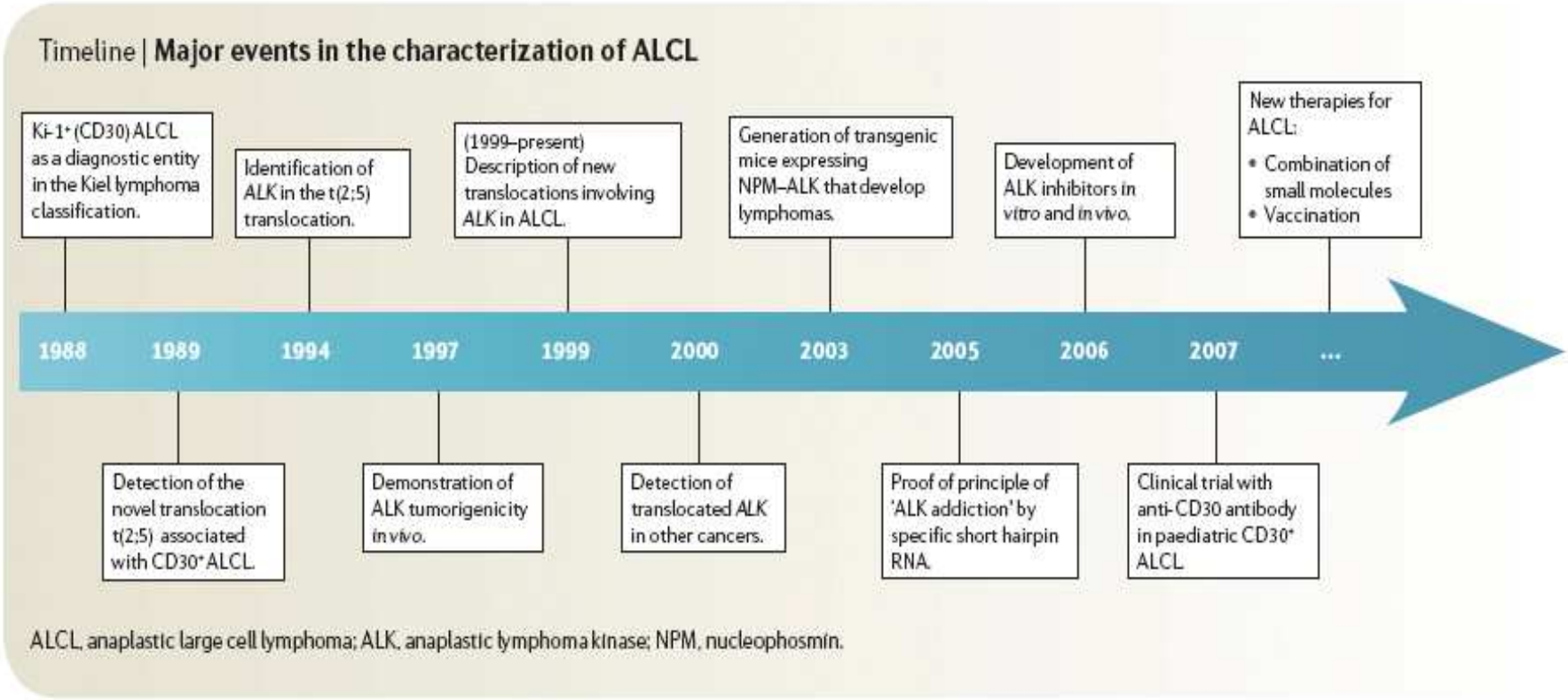






Fusion of a kinase gene, ALK, to a nuclear protein gene, NPM, in non-Hodgkin's lymphoma

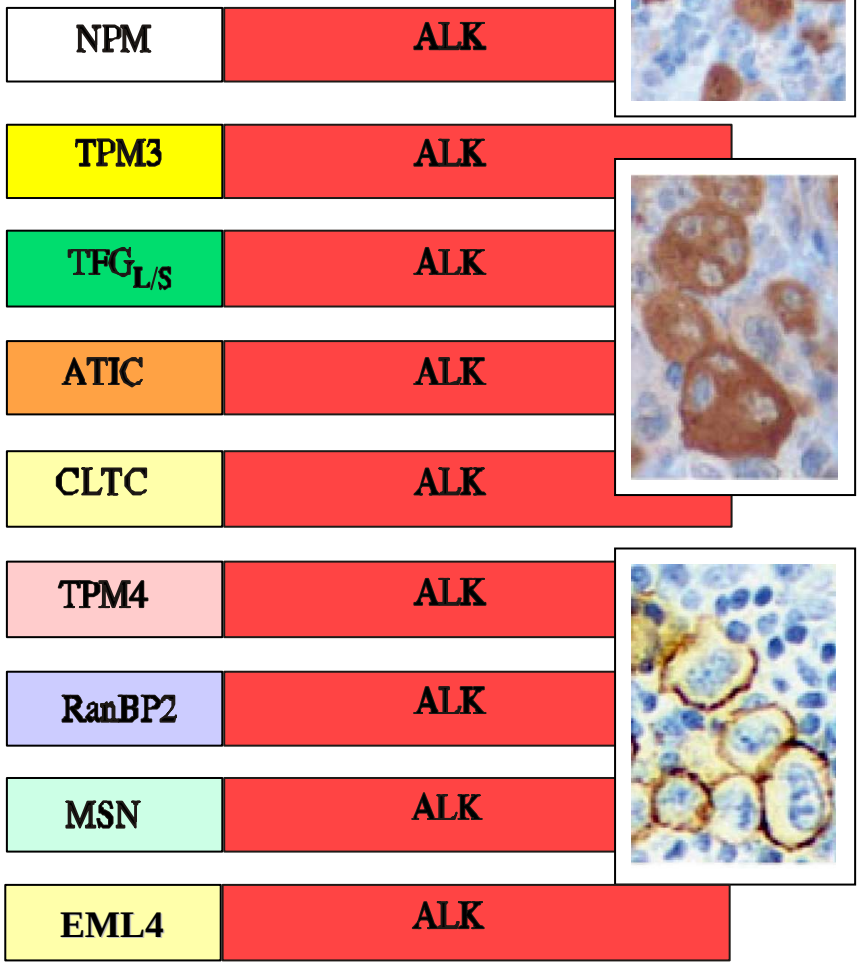
Morris S.W. et al. *Science* 1994;263(5151):1281-4

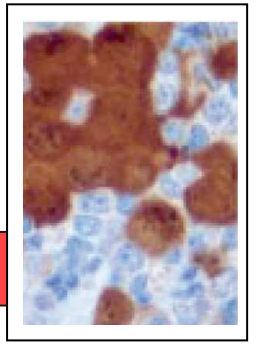


Chiarle et al., Nature Rev Cancer 2008

Translocations and fusion proteins involving the ALK gene in ALCL

Translocation	Frequency (NHL)	Localization
t(2;5)(p23 ;q35)	70-80%	Cytoplasmic/Nuclear nucleolar
t(1;2)(q25 ;p23)	10-20%	Cytoplasmic
t(2;3)(p23 ;q21)	2-5%	Cytoplasmic
inv(2)(p23 ;q35)	2-5%	Cytoplasmic
t(2;17)(p23 ;q23)	2-5%	Cytoplasmic
t(2;19)(p23 ;q13,1)	-	Cytoplasmic
t(2;2)(p23 ;q11-13)? or inv(2)(p23 ;q11-13)?	-	Nuclear membrane
t(X;2)(q11-12 ;p23)	-	Cell-Membrane
t(2;17)(p23 ;q25)	2-6% (**NSCLC**)	Cytoplasmic



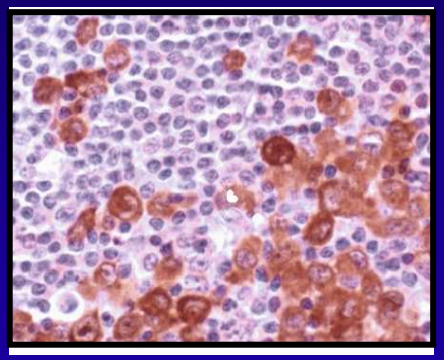


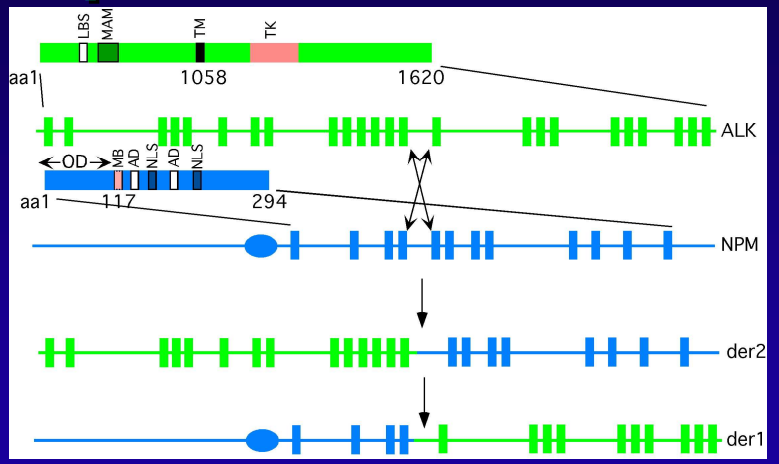
Chromosomal translocations involving anaplastic lymphoma kinase gene in cancers

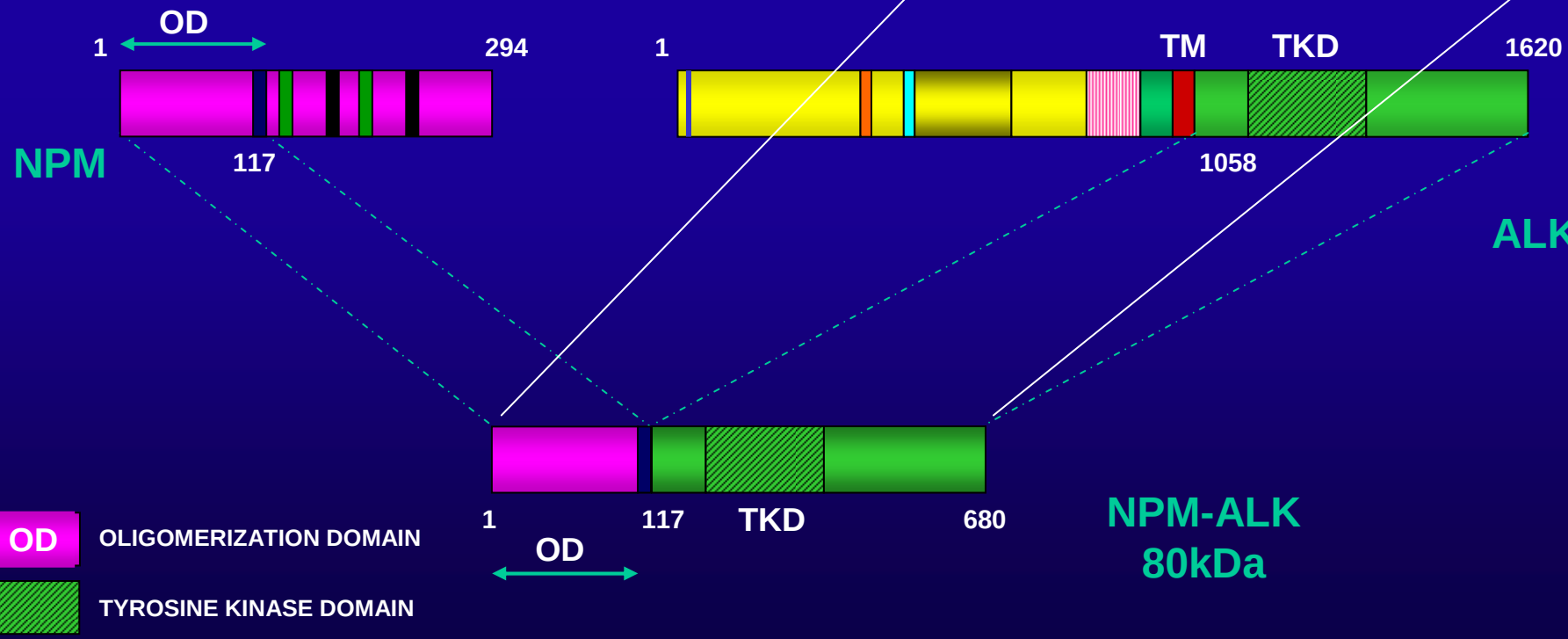
Disease	Fusion protein	Chromosomal abnormality	Principal references
ALCL	NPM–ALK	t(2;5)(p23;q35)	Morris *et al.* (1994) and Shiota *et al.* (1994)
ALCL	ALO17–ALK	t(2;17)(p23;q25)	Cools *et al.* (2002)
ALCL	TFG–ALK	t(2;3)(p23;q21)	Hernández *et al.* (1999, 2002)
ALCL	MSN–ALK	t(2;X)(p32;q11–12)	Tort *et al.* (2001, 2004)
ALCL	TPM3–ALK	t(1;2)(q25;p23)	Lamant *et al.* (1999) and Siebert *et al.* (1999)
ALCL	TPM4–ALK	t(2;19)(p23;p13)	Meech *et al.* (2001)
ALCL	ATIC–ALK	inv(2)(p23;q35)	Colleoni *et al.* (2000), Ma *et al.* (2000), and Trinei *et al.* (2000)
ALCL	MYH9–ALK	t(2;22)(p23;q11·2)	Lamant *et al.* (2003)
ALCL	CLTC–ALK	t(2;17)(p23;q23)	Touriol *et al.* (2000)
IMT	TPM3–ALK	t(1;2)(q25;p23)	Lawrence *et al.* (2000)
IMT	TPM4–ALK	t(1;19)(p23;p13)	Lawrence *et al.* (2000)
IMT	CLTC–ALK	t(2;17)(p23;q23)	Bridge *et al.* (2001) and Patel *et al.* (2007)
IMT	ATIC–ALK	inv(2)(p23;q35)	Debiec-Rychter *et al.* (2003)
IMT	SEC31L1–ALK	t(2;4)(p23;q21)	Panagopoulos *et al.* (2006)
IMT	RANBP2–ALK	t(2;2)(p23;q13) inv(2)(p23;p15;q31)	Ma *et al.* (2003)
IMT	CARS–ALK	t(2;11;2)(p23;p15;q31)	Cools *et al.* (2002) and Debelenko *et al.* (2003)
NSCLC	EML4–ALK	inv(2)(p21;p23)	Rikova *et al.* (2007) and Soda *et al.* (2007)
NSCLC	TFG–ALK	t(2;3)(p23;q21)	Rikova *et al.* (2007)
DLBCL	NPM–ALK	t(2;5)(p23;q35)	Adam *et al.* (2003) and Onciu *et al.* (2003)
DLBCL	CLTC–ALK	t(2;17)(p23;q23)	De Paepe *et al.* (2003)
DLBCL	Unknown	ins(3'ALK)(4q22–24)	Stachurski *et al.* (2007)
DLBCL	SQSTM1–ALK	t(2;5)(p23·1;q35·3)	Takeuchi *et al.* (2010)
DLBCL	SEC31A–ALK	ins(4)(2;4)(?;q21) t(2;4)(p24;q21)	Bedwell *et al.* (2010) and Van Roosbroeck *et al.* (2010)
SCC	TPM4–ALK	t(2;19)(p23;p13)	Du *et al.* (2007) and Jazii *et al.* (2006)
RCC	VCL–ALK	t(2;10)(p23;q22)	Debelenko *et al.* (2010)

NPM-ALK chimeric protein

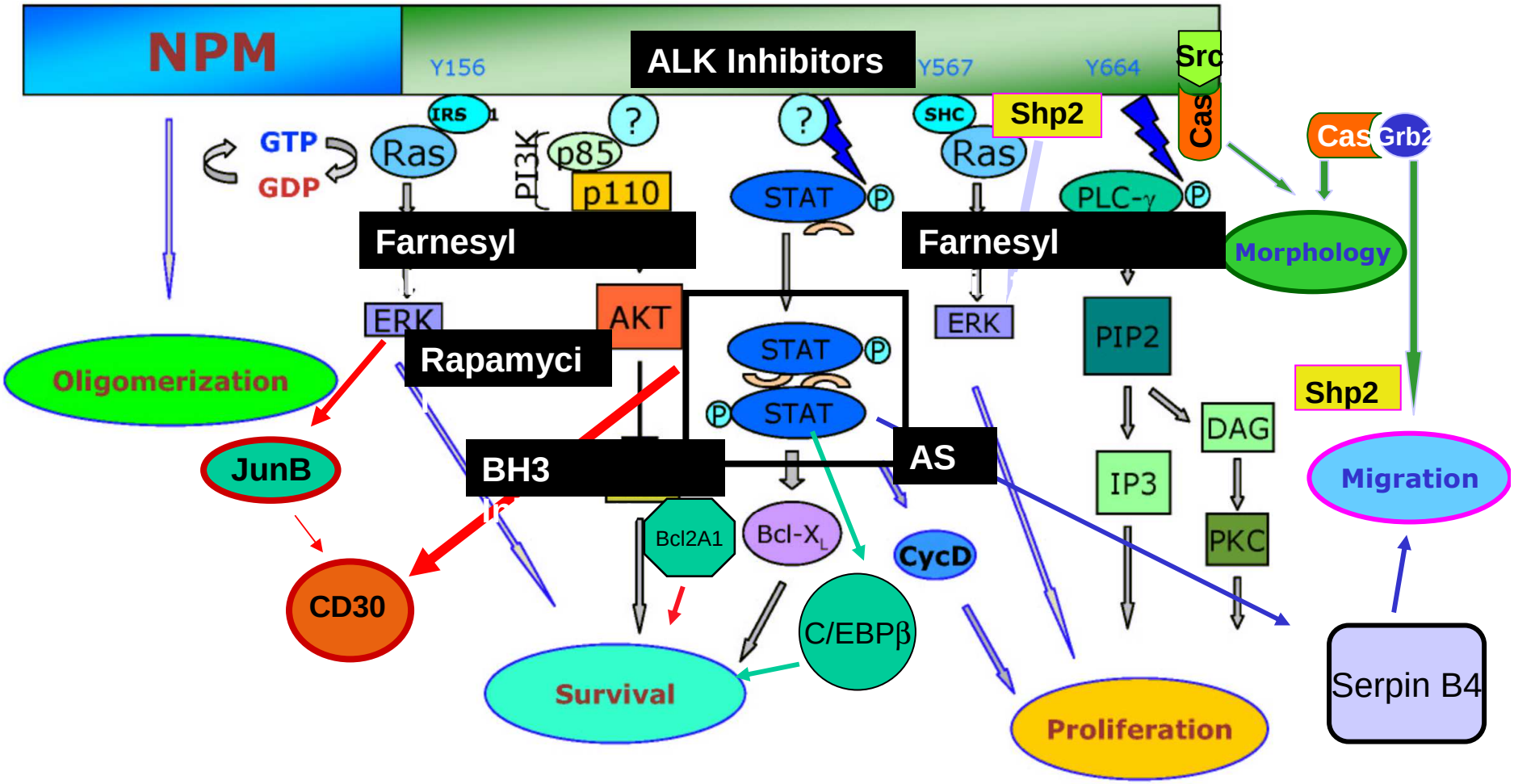
ALCL



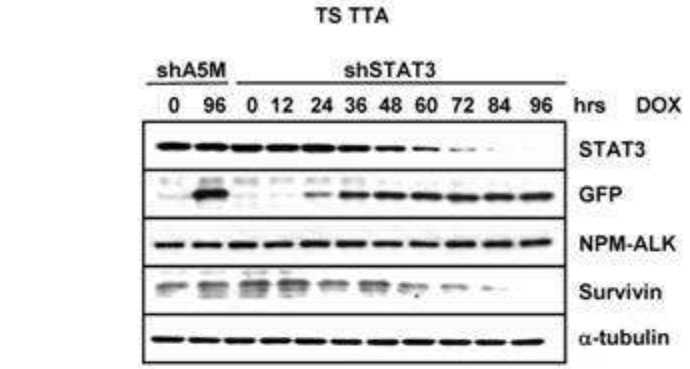


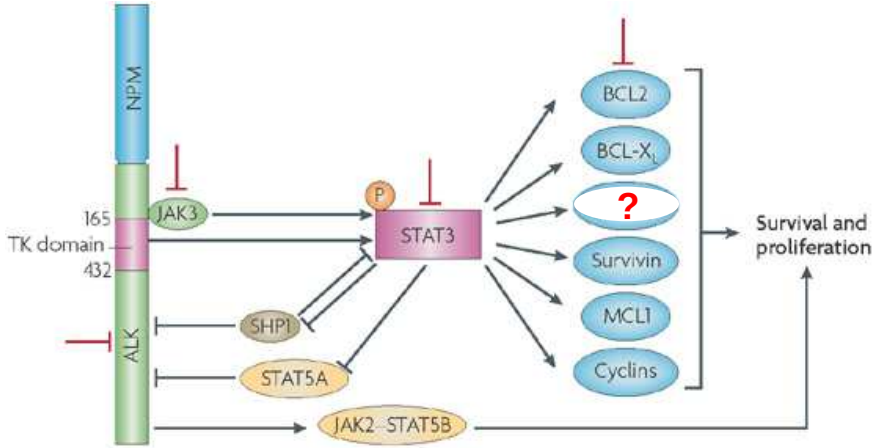


Oncogenic signaling cascade activated by NPM-ALK

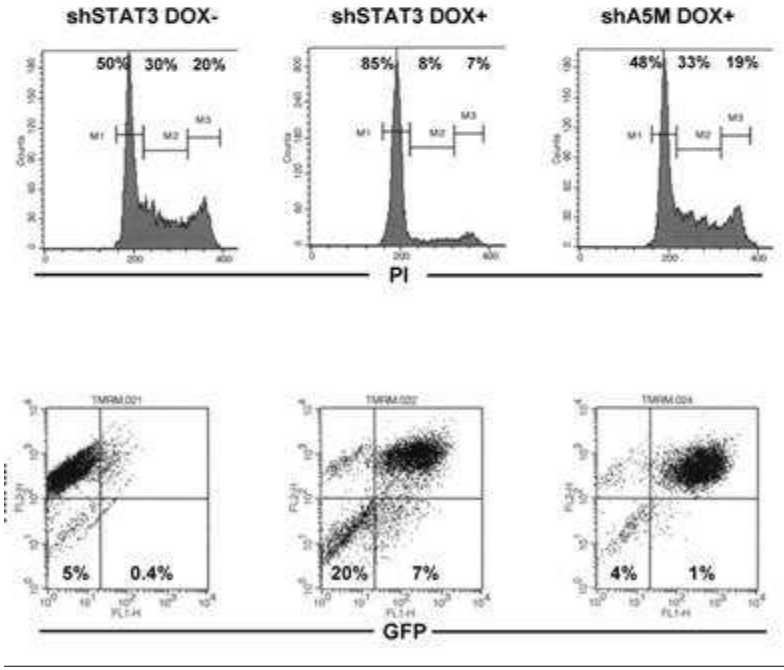


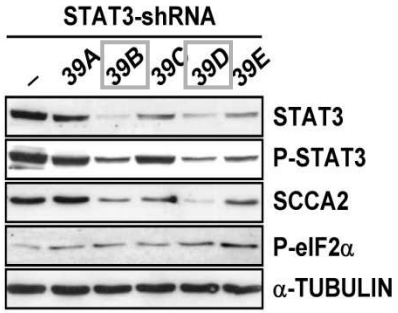
STAT3 silencing induces cell cycle arrest and apoptosis

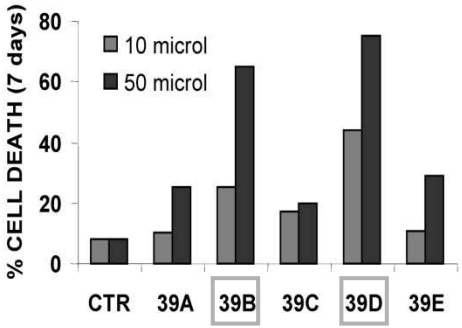




Chiarle et al., 2008 Nature Reviews | Cancer

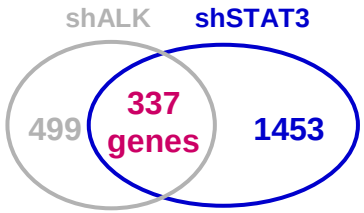




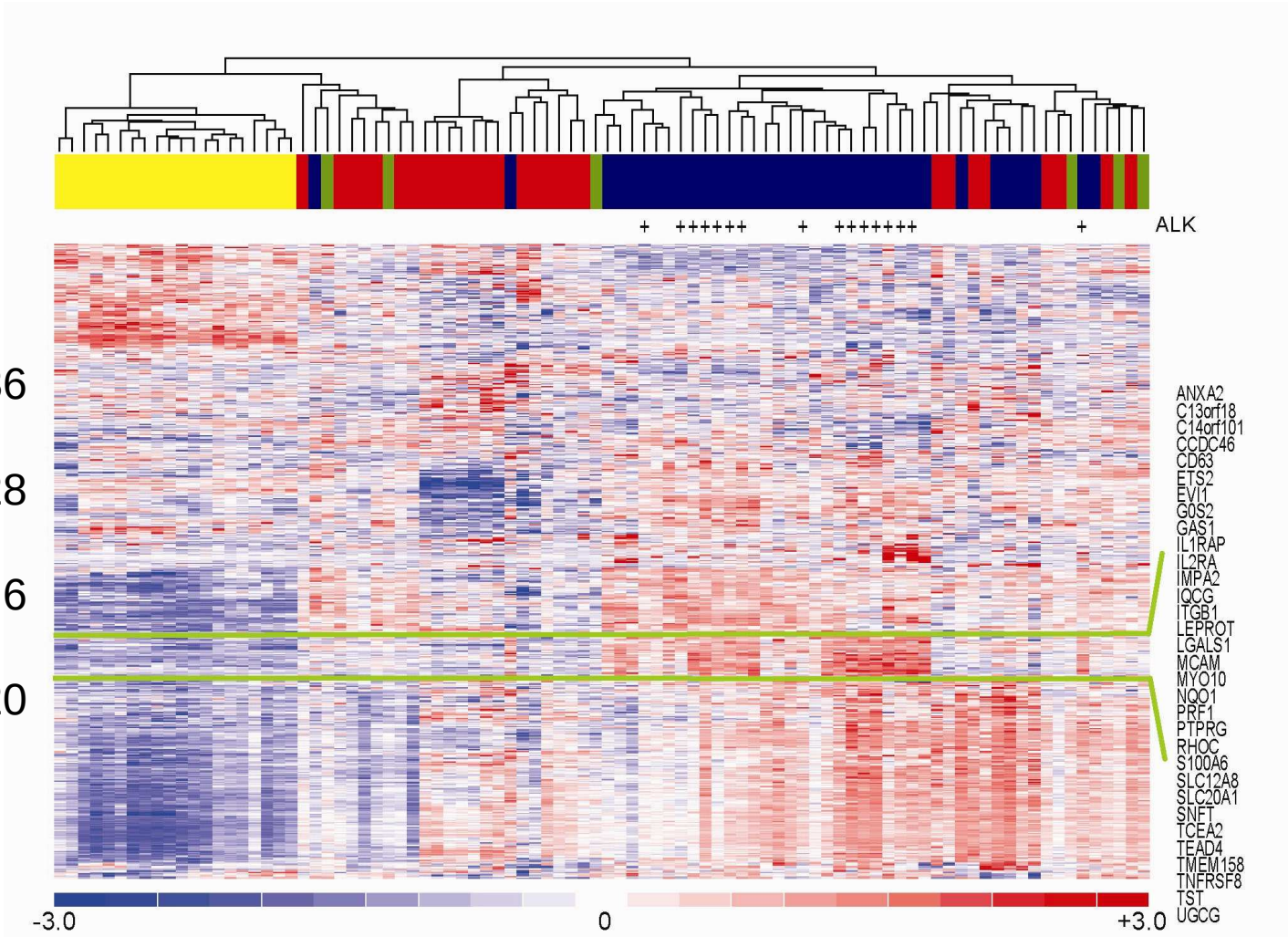


Piva et al. JC0, in press

ALK/STAT3 signature predicts ALK status in T-NHL patients



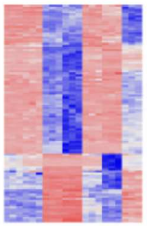
ALCL	36
PTCL-NOS	28
AILT	6
T-Cells	20



Biological validation of new NPM-ALK putative targets

Inducible/drug specific NPM-ALK KO in ALCL cells and/or primary ALCL

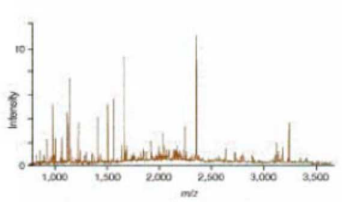
Gene expression profile



Up-regulated

Down-regulated

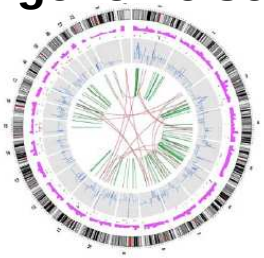
Proteomic



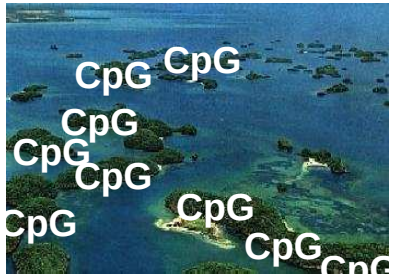
Substrates

Interactors

All genome seq



DNA methylation



X number of candidates (A, B, C…Z)

Generation of 2 libraries

shRNA

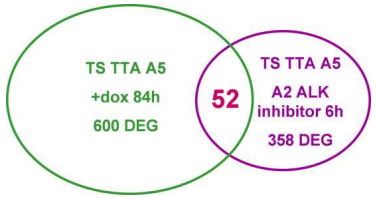
cDNA

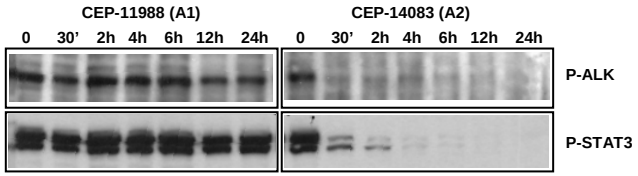
Transduction in ALCL cells with inducible NPM-ALK KD

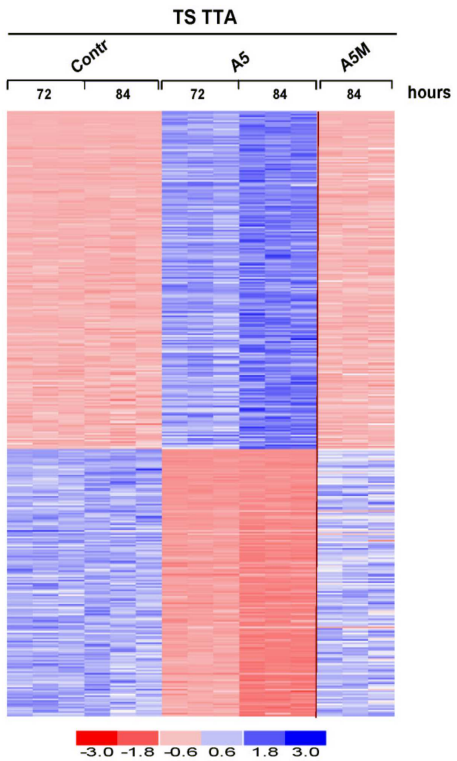
NPM-ALK ON + A shRNA → Cell growth arrest - apoptosis

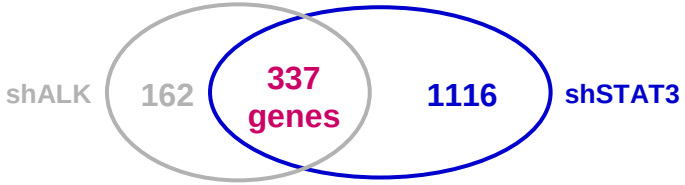
NPM-ALK KD + A cDNA → Partial rescue of the phenotype

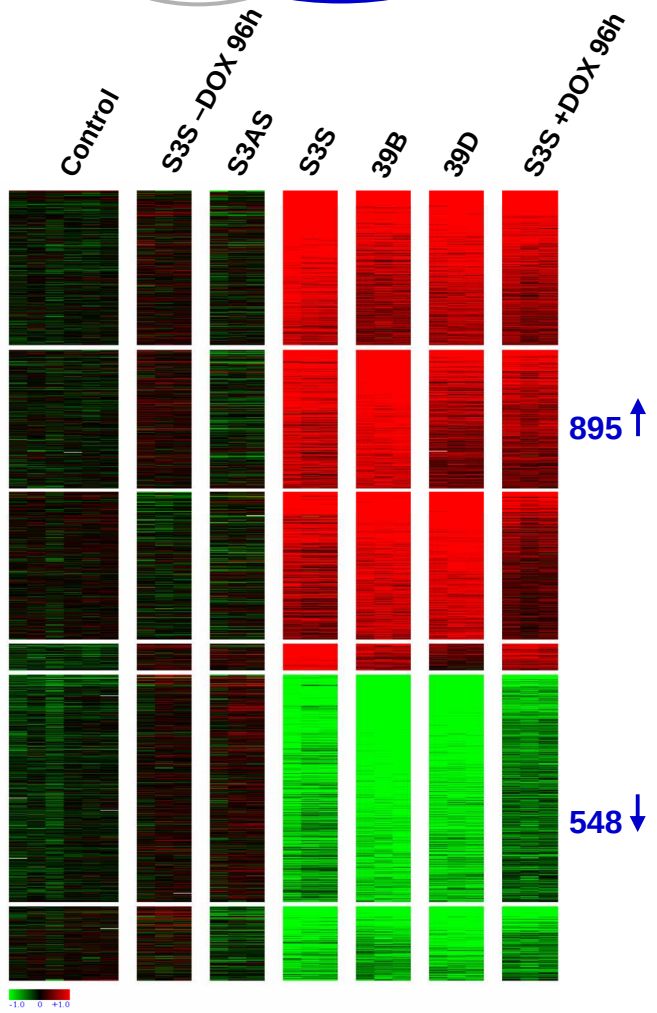
ALK expression signature is largely dependent upon STAT3 activity





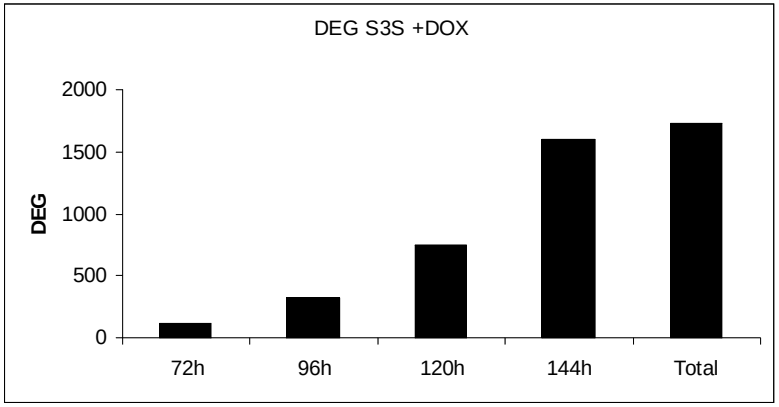






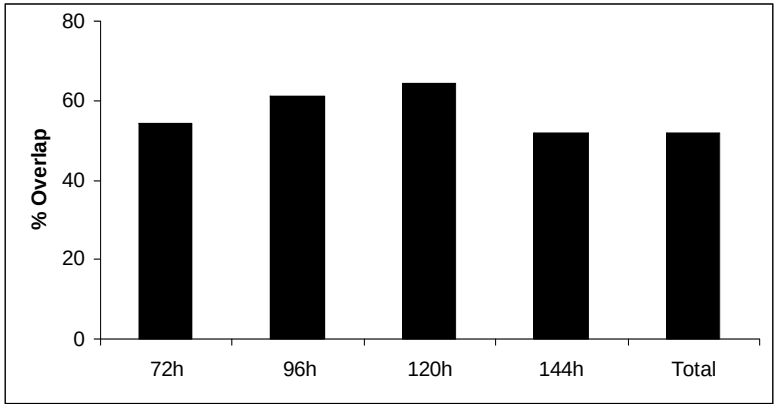
The kinetics of STAT3-regulated genes

Platform: Illumina HumanHT-12 BeadChip (25,000 genes)
Differential Score: p<0.001
FC>2

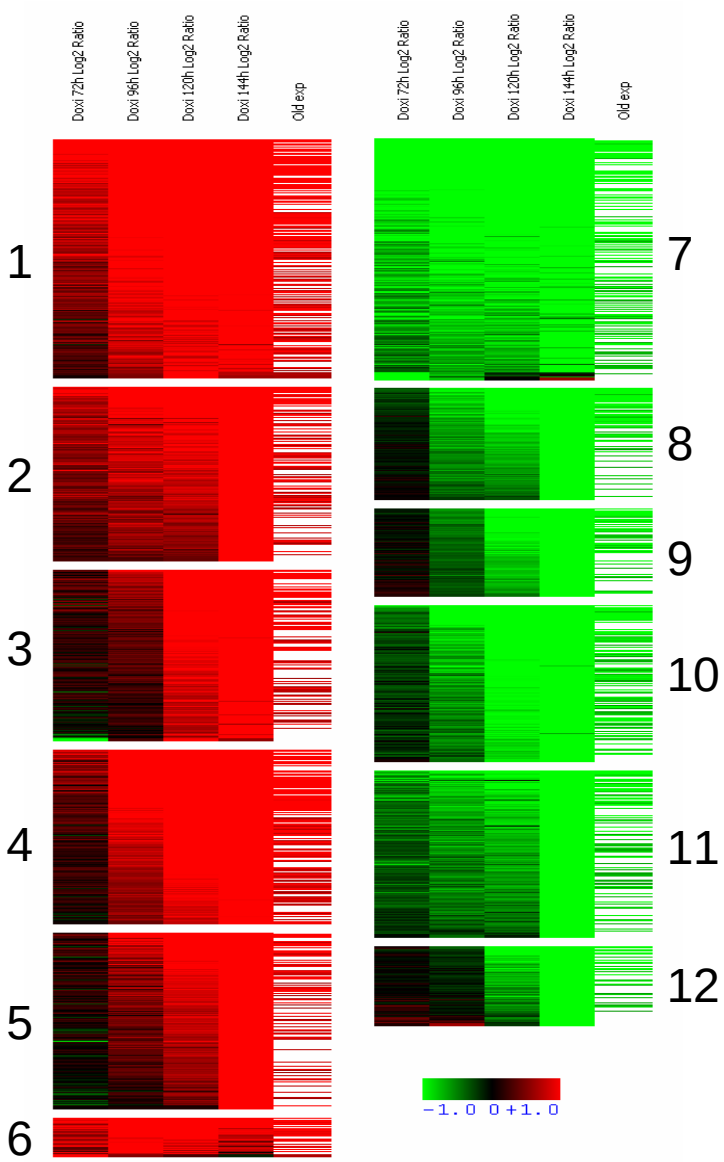


DEG at least in one time-point 1730

Overlap with a previous STAT3 KD GEP experiment performed with 3 different shRNA at 84-96h



Clustering



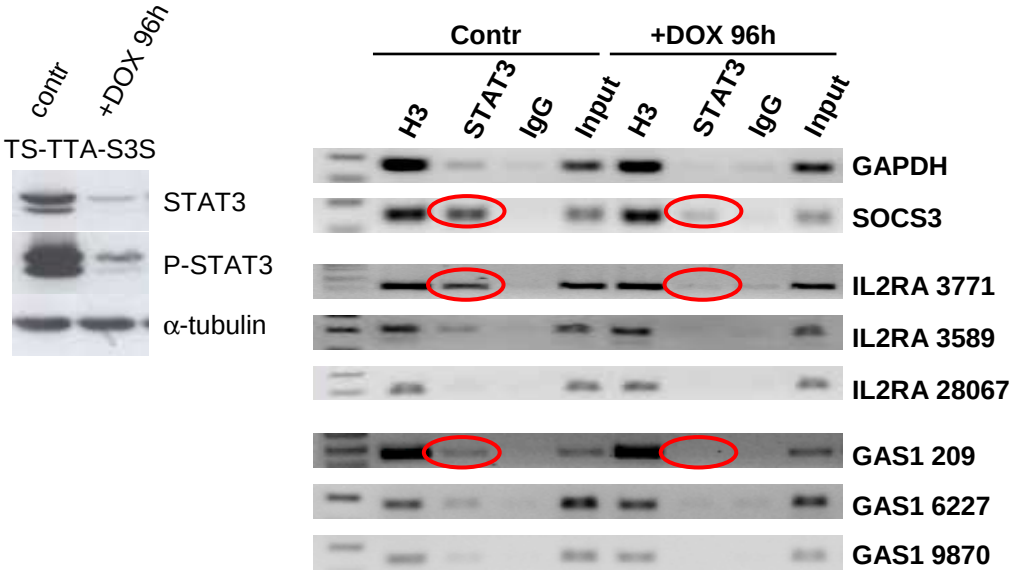
Identification and validation of STAT3 binding sites

Position Weight Matrix approach

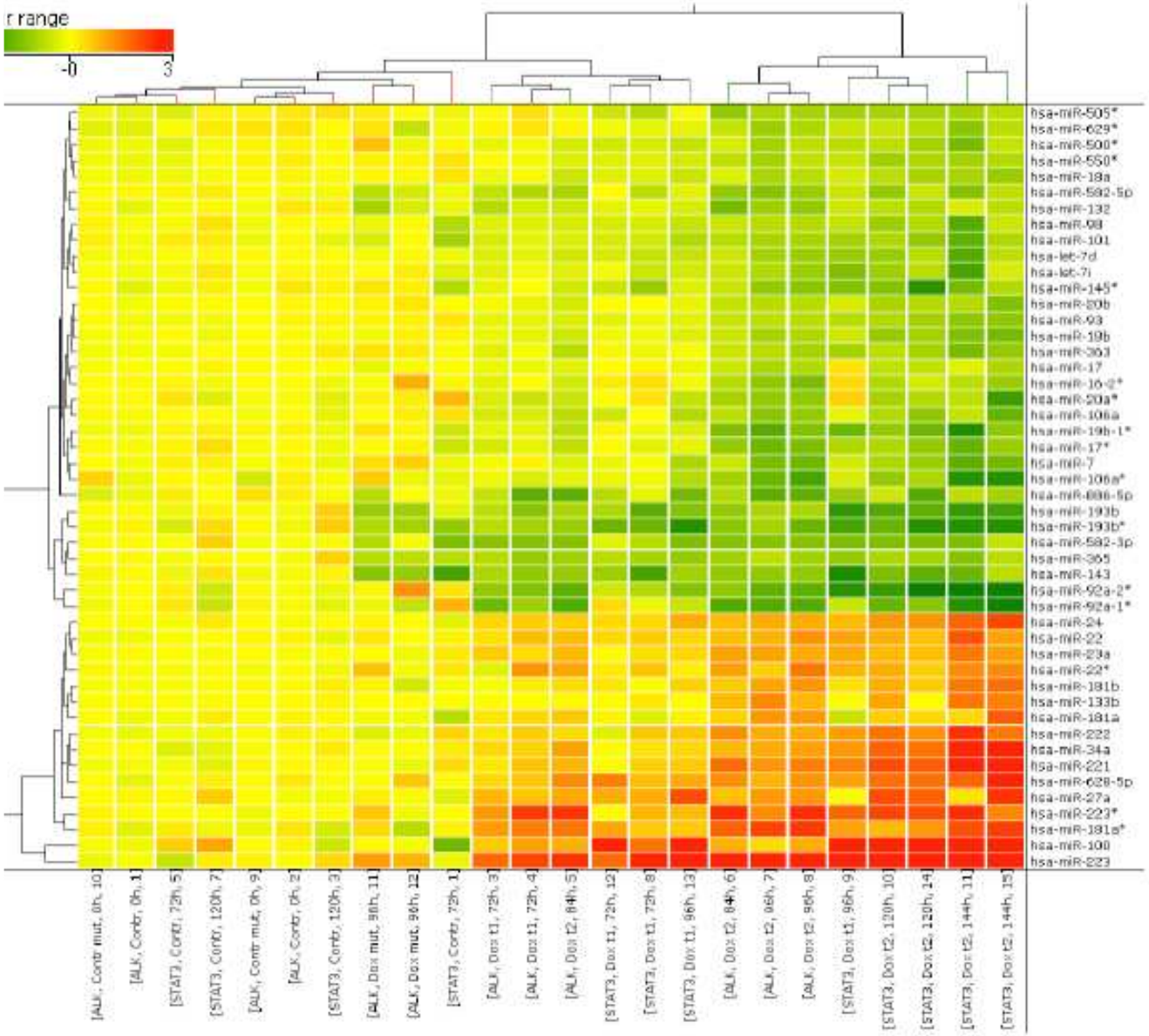
STAT3 CONSENSUS SITES

GENE	POSITION	SCORE	CONSERVATION
TNFRSF8	-3219	10,18	Bt
	-1257	10,28	Cf
	-374	*	
	-20	12,96	
	19238	9,94	Bt
GAS1	-209	14,53	
	-6227	10,24	
	-9870	11,11	
SCCA2	-1538	10,28	
	-2885	10,47	
	-2963	10,28	
	-3011	9,95	
	-4159	11,87	
	-6224	10,47	
ICOS	-2448	10,81	Bt
	1815	10,47	Mm
	12765	10,24	Cf, Bt
	14486	10,58	Bt
IL2RA	-3771	11,11	Cf, Bt
	472	11,87	Bt
	3589	11,11	Mm, Cf, Bt
	11008	9,87	Cf, Bt
	23677	9,95	Bt
	25962	11,87	Cf
	28067	12,96	Cf, Bt
HSD17B7	-6685	9,72	Cf
	-3473	10,91	Cf
SGK	13146	10,18	Mm
	39382	10,22	Mm, Bt
RGS16	-6374	10,58	Mm, Cf, Bt
	-4789	9,95	Bt
	-2251	10,81	Cf, Bt
	941	12,96	Bt

Validation of STAT3 binding sites by CHIP

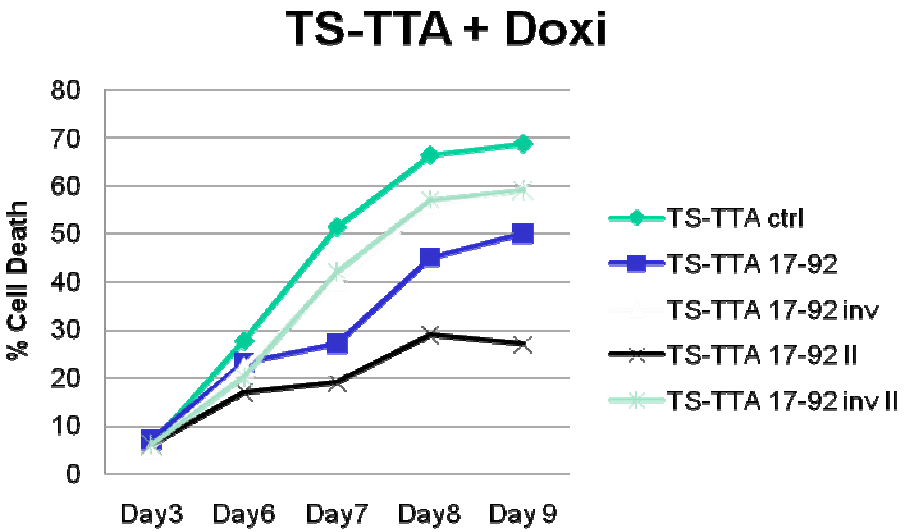


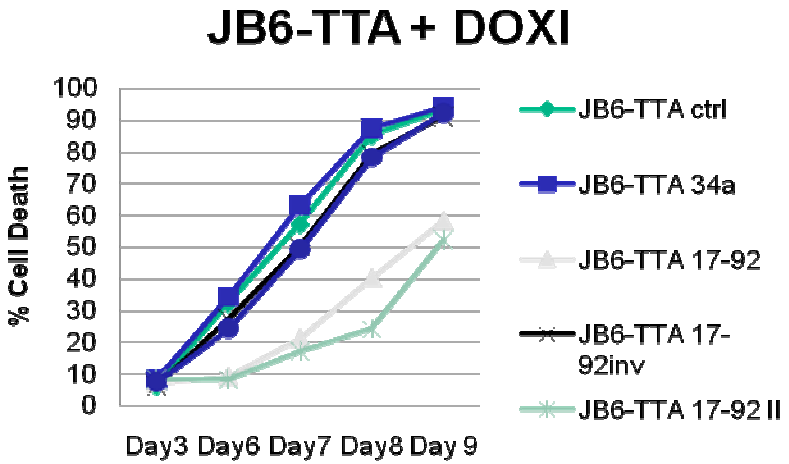
microRNA EXPRESSION PROFILING FOLLOWING INDUCIBLE ALK OR STAT3 KNOCK DOWN IN ALK+ ALCL CELLS



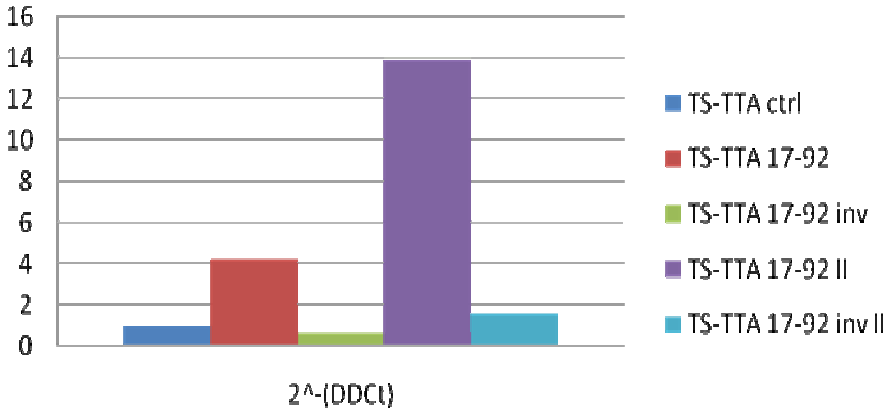
Name	p-value	FC Absol	regulation
let-7d	4,81E-04	1,9	down
let-7i	0,004114005	1,9	down
miR-100	0,013155356	6,4	up
miR-101	0,003949106	1,8	down
miR-132	3,75E-04	1,9	down
miR-133b	0,004847618	1,8	up
miR-16-2*	1,93E-04	1,9	down
miR-22	2,08E-04	2,1	up
miR-22*	1,08E-04	2,0	up
miR-223	1,01E-04	18,0	up
miR-223*	7,25E-06	4,2	up
miR-34a	4,66E-04	3,0	up
miR-500*	7,76E-04	1,7	down
miR-505*	4,31E-04	1,8	down
miR-550*	8,68E-05	1,8	down
miR-582-3p	0,001124796	2,2	down
miR-582-5p	5,10E-05	2,1	down
miR-628-5p	8,96E-05	2,9	up
miR-629*	0,002917039	1,7	down
miR-7	4,44E-04	2,3	down
miR-886-5p	3,92E-04	2,5	down
miR-93	1,08E-04	1,9	down
miR-98	0,007044873	1,7	down
miR-106a	1,08E-04	2,1	down
miR-106a*	0,001166221	2,8	down
miR-20b	7,25E-05	1,8	down
miR-18b	1,02E-04	2,0	down
miR-92a-2*	7,62E-05	4,7	down
miR-363	7,25E-05	2,0	down
miR-17	1,34E-04	1,6	down
miR-17*	1,02E-04	2,3	down
miR-18a	9,31E-05	1,8	down
miR-19b-1*	1,68E-04	2,7	down
miR-20a*	8,44E-04	2,3	down
miR-92a-1*	5,33E-05	4,2	down
miR-181a	0,003438643	1,9	up
miR-181a*	7,62E-05	3,5	up
miR-181b	4,90E-04	2,0	up
miR-193b	1,68E-04	3,0	down
miR-193b*	5,86E-04	3,2	down
miR-365	2,31E-04	2,0	down
miR-143	0,012849905	2,0	down
miR-145*	0,003508412	2,2	down
miR-221	1,68E-04	3,6	up
miR-222	5,55E-04	2,4	up
miR-23a	5,10E-05	2,0	up
miR-24	3,30E-04	2,3	up
miR-27a	0,001675232	2,4	up

Mir17-92 overexpression rescues STAT3 KD of ALK+ ALCL cells

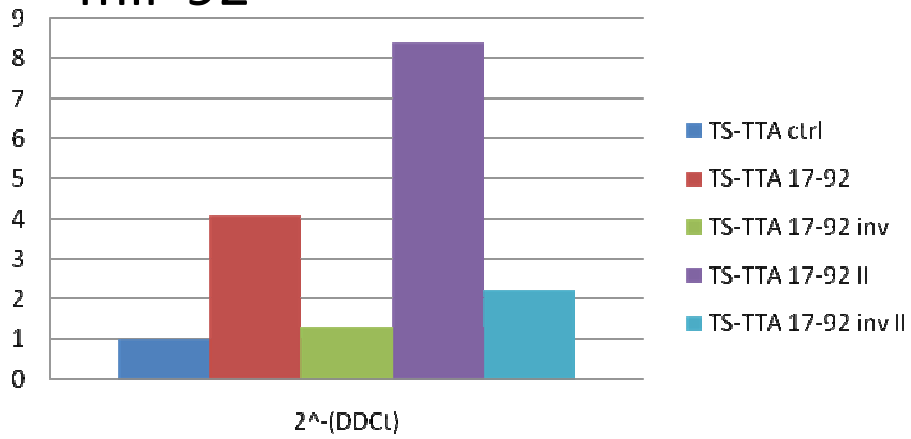




TS-TTA qRT-PCR: primer mir-19a



TS-TTA qRT-PCR: primer mir-92



Strategies and Challenges to Therapeutic Intervention into STAT3 Signaling

Strategy	Targets	Examples	Challenges
Inhibit phosphorylation/ activation of STAT3	EGFR agonism TKR activity JAK activity SFK activity	Cetuximab, panitumumab Gefitinib, erlotinib, lapatinib AG490, LS-104, ICNB1824, CEP-701 Dasatinib, AZD0530, bosutinib	Modest efficacy; development of resistance; myelosuppression, GI toxicity, and adverse events; kinase selectivity and cardiovascular toxicity
Inhibit intermolecular interactions that involve STAT3	STAT3 SH-2 domains	Oligopeptides designed from EGFR, gp130, and other receptor or pY-containing peptides; peptide aptamers; G-quartet oligonucleotides; small-molecule peptidomimetics	Poor cell permeability and efficacy; poor metabolic stability; poor selectivity for specific SH2 domains; potential for adverse events
Inhibit nuclear import/ export of STAT3	Importins α3, α 5, α 7 Importin β Exportin 1	Karyostatin 1A (effect on STAT3 undetermined) Leptomycin B and Ratjadone A	Multicomponent nature of nuclear pore and translocation not fully determined; specificity for translocated proteins problematic
Inhibit STAT3-mediated transcription	DNA binding site of STAT3	dsODN decoys; peptide aptamers	Poor cell permeability without effective and specific delivery systems; poor metabolic stability
Natural products	Unspecified	Guggulsterone, honokiol, curcumin, resveratrol, flavopiridol, cucurbitacin	Specificity, potency, and efficacy, mechanism of action unknown

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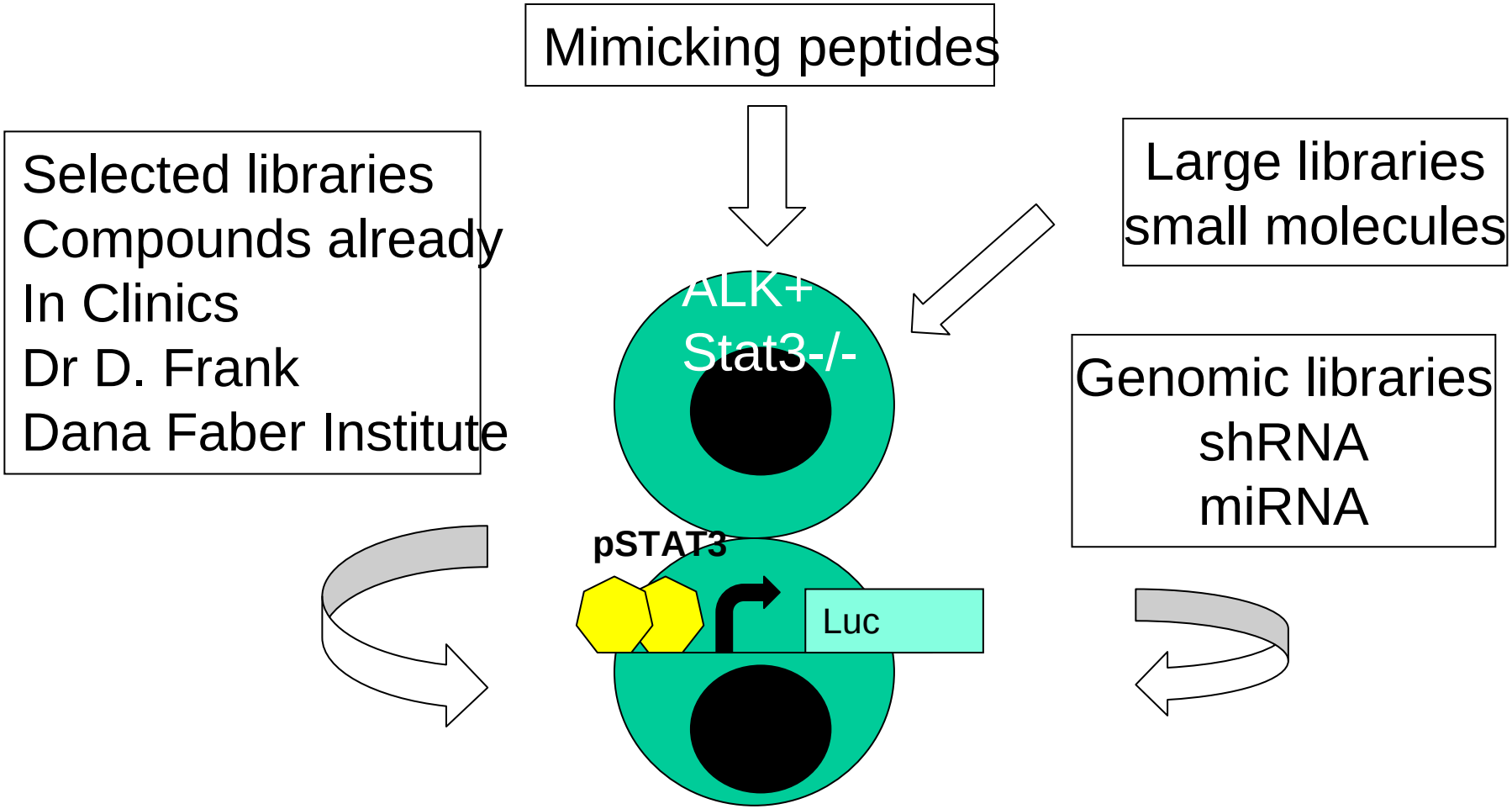
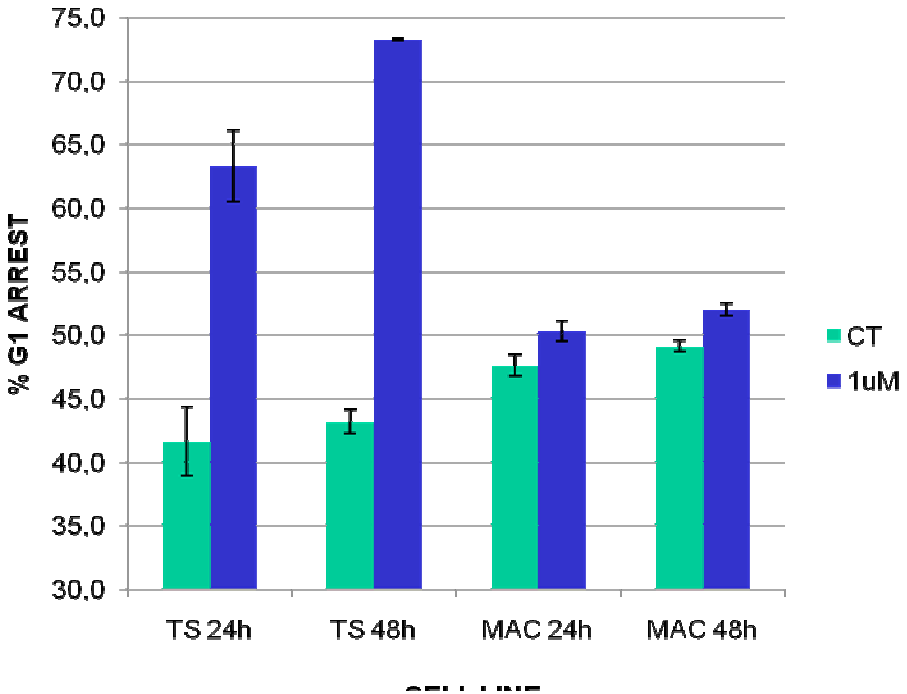
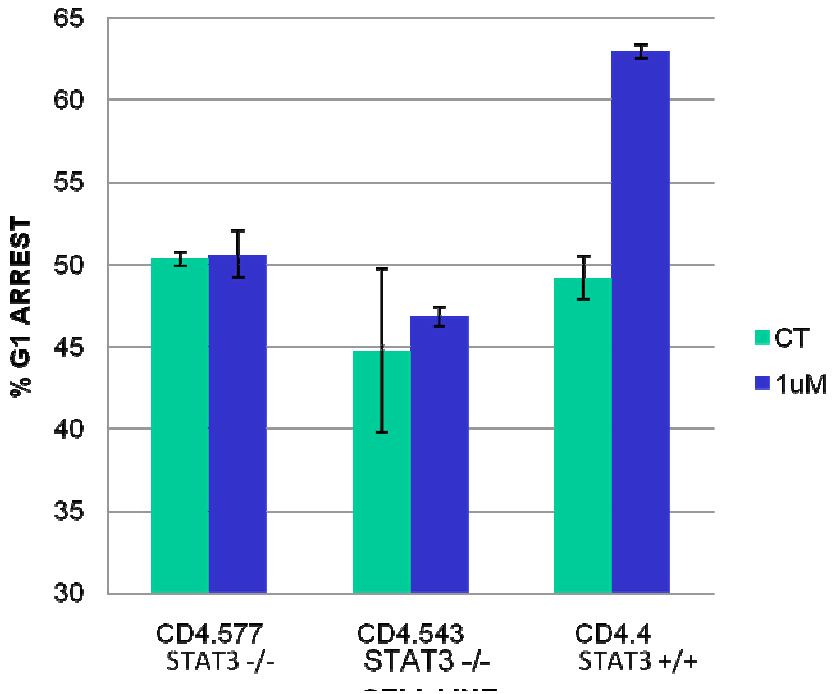
Drug discovery strategies



How should we select the right models to test STAT3 inhibitors?



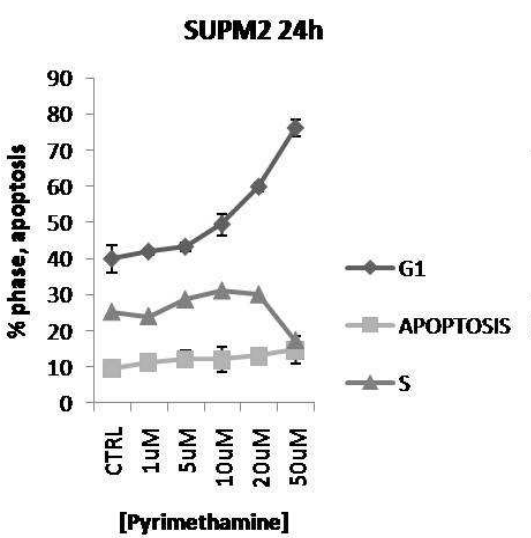


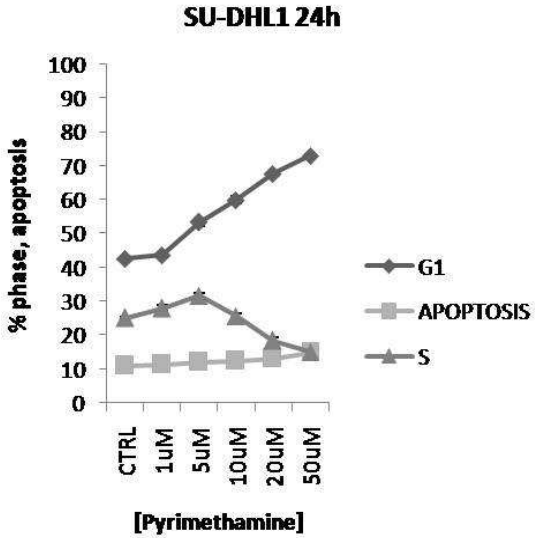
ALK	+	-	+	+
STAT3	+	+	-	-
+				
STAT5	-	+	-	-

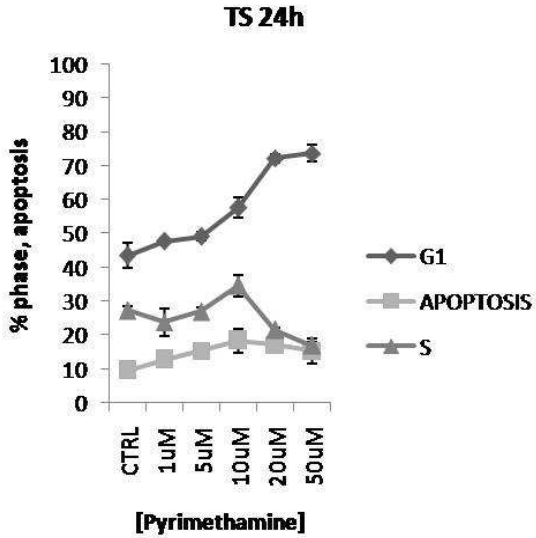
Cortese D.

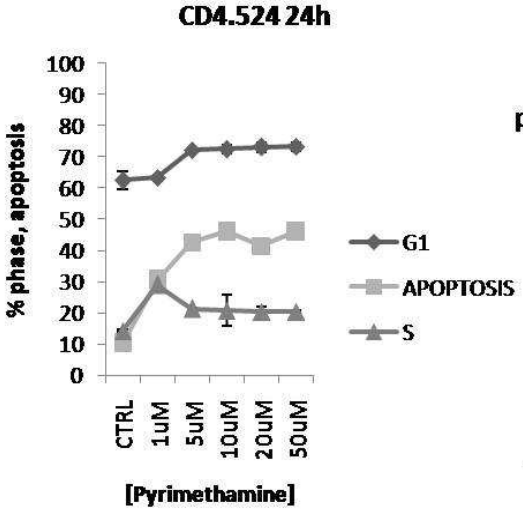
PYRIMETHAMINE

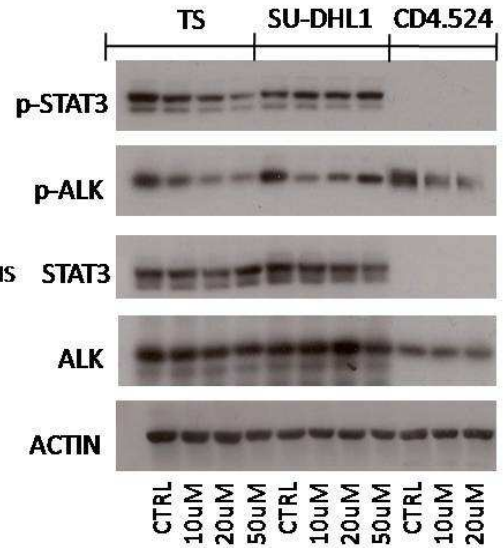
STAT3 INHIBITORS

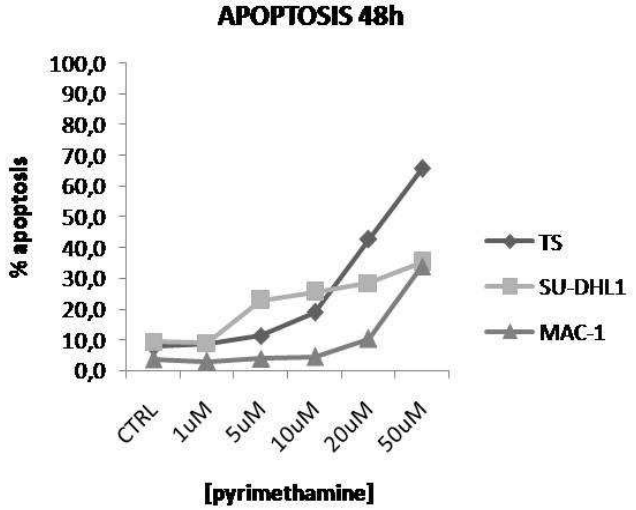












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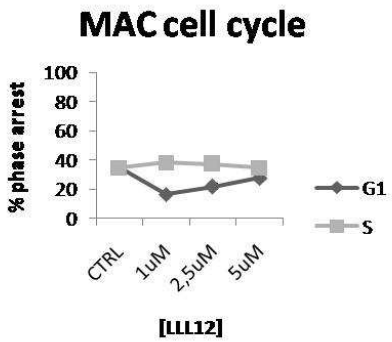
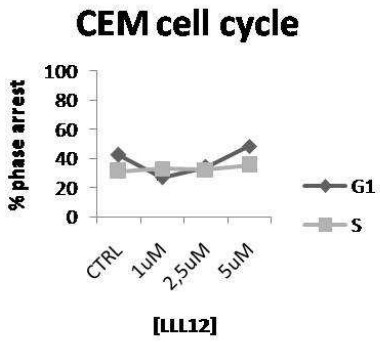
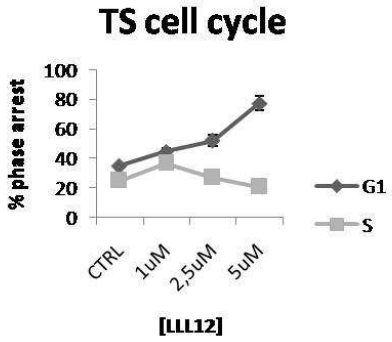
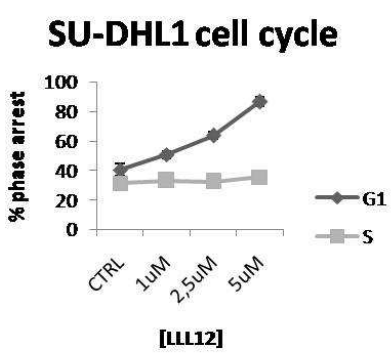
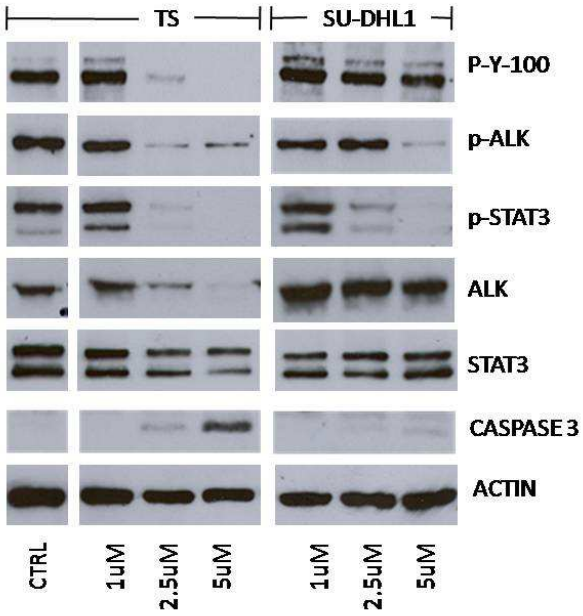
STAT3 INHIBITORS

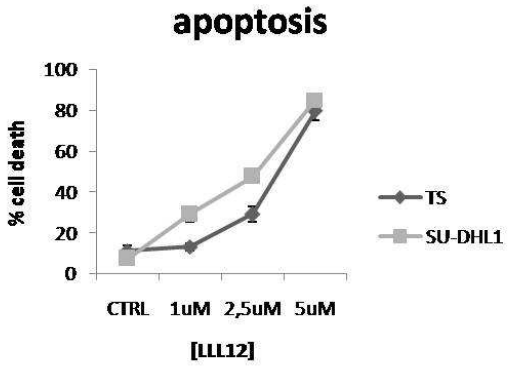


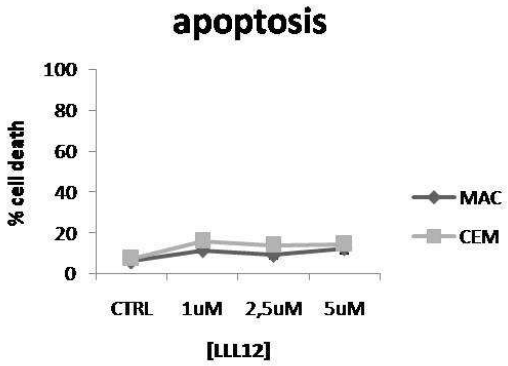








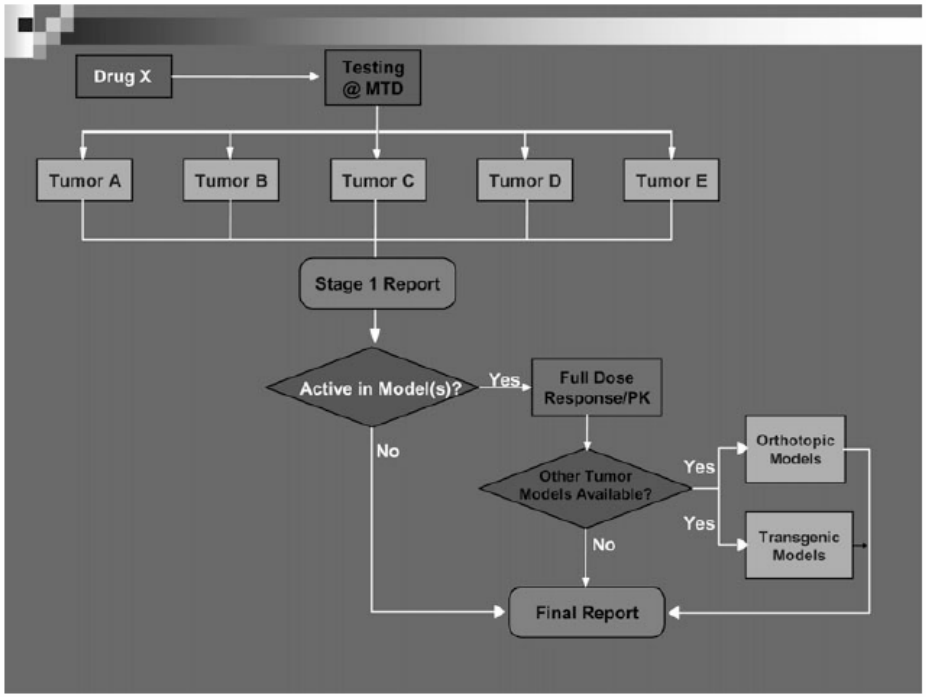




From Human to Mouse and Back: "Tumorgraft" Models Surge in Popularity

By Ken Garber

Mouse xenograft models of cancer, understandably, have a terrible reputation. Although researchers and companies routinely use these human tumors in mice for preclinical drug testing, individual models poorly predict how drugs will act in the clinic. Retrospective reviews published by the National Cancer Institute in 2001 and the National Cancer Institute of Canada in 2003 came to the same conclusion: Drugs that work against cancer in xenograft mice rarely work in people with the same tumor,



New drug testing in mouse models by the NCI-supported Pediatric Preclinical Testing Program. The 3-year-old program, which has already sent several drugs into clinical trials

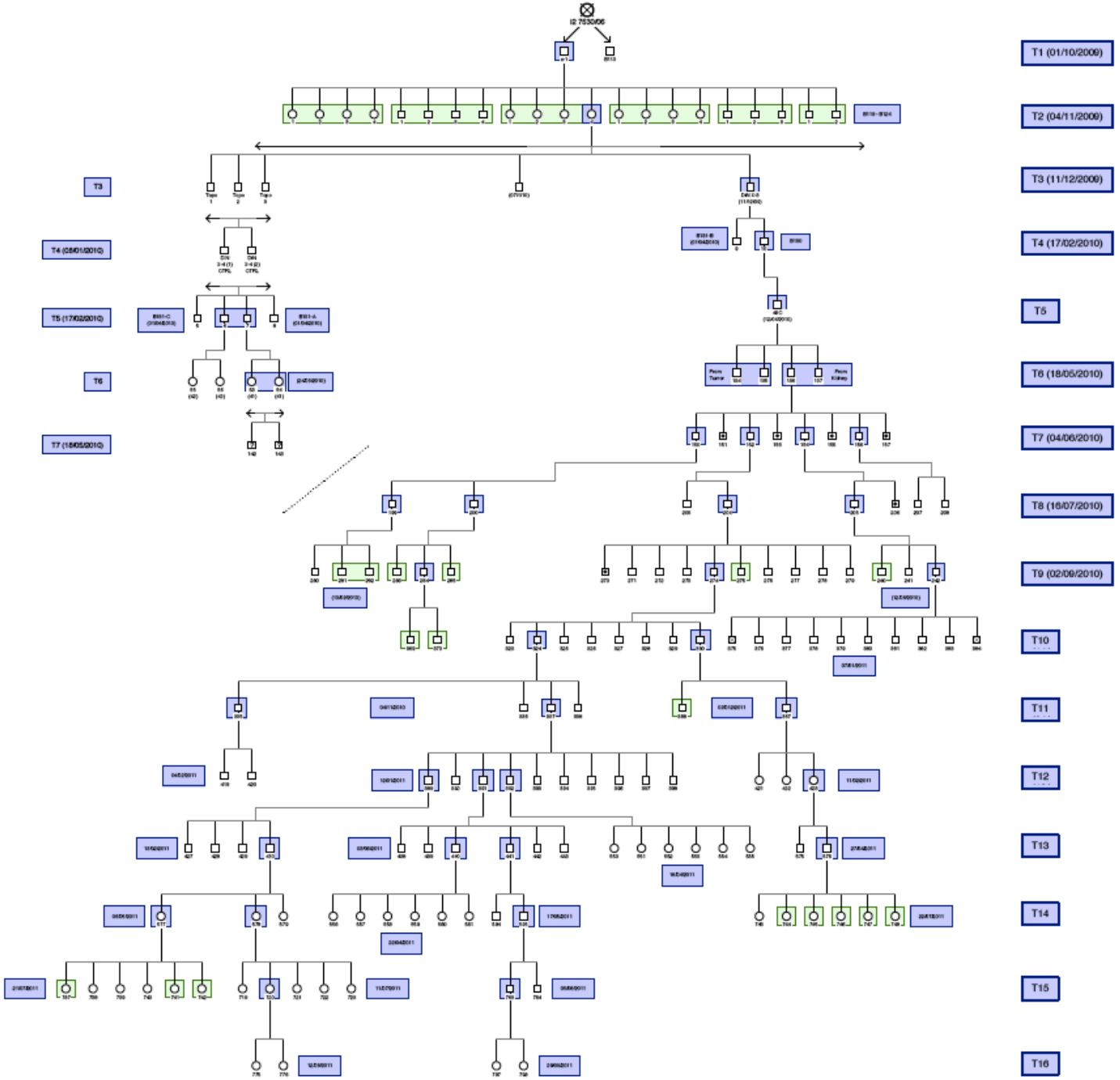
resistance to gemcitabine, the standard therapy for pancreatic cancer, and sensitivity to mitomycin C, a rarely used treatment. The patient—who first failed gemcitabine therapy—received mitomycin C and has been in remission for more than 2 years.

The trial's principal investigator, Manuel Hidalgo, M.D., Ph.D., stressed that tumorgrafts, at present, can be of only limited use for individualizing patient treatment because of the time and resources necessary to create tumorgraft banks. Many

Where are we going with novel personalized cancer strategies?

Comparative Oncology Program

NATIONAL CANCER INSTITUTE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T3 (11/12/2009)
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T5
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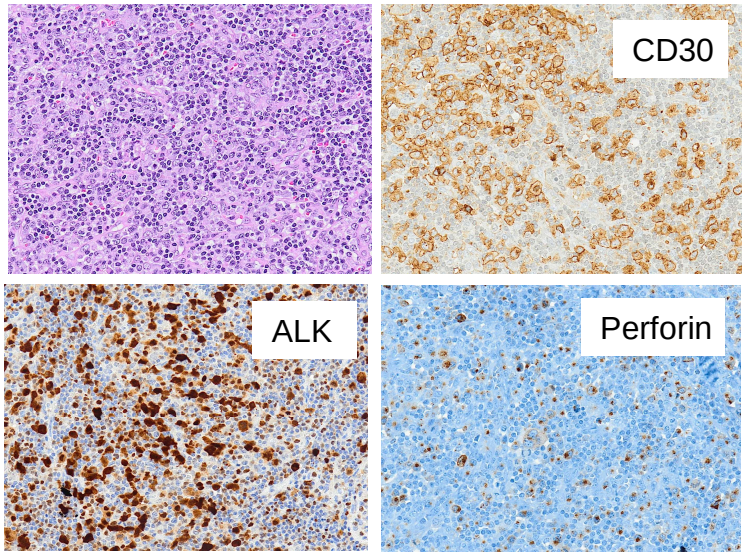
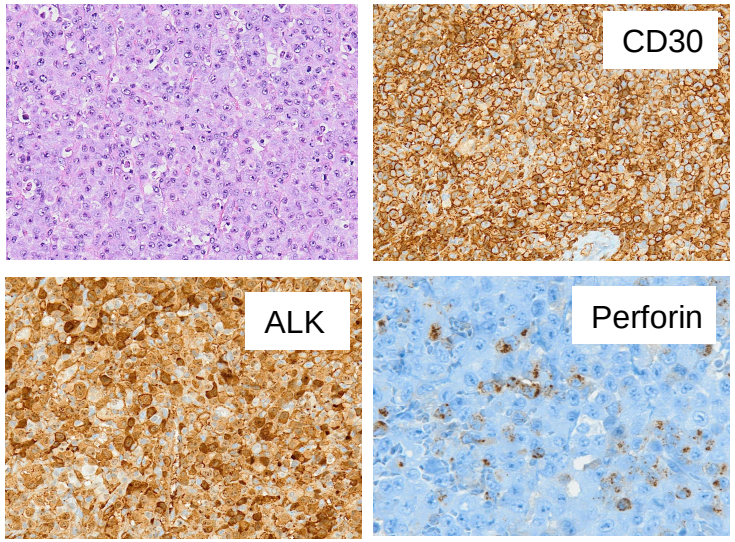
T8 (16/07/2010)
T9 (02/09/2010)
T10
T11
T12
T13
T14
T15
T16
T3
T4 (08/01/2010)
T5 (17/03/2010)
T6
T7 (18/05/2010)

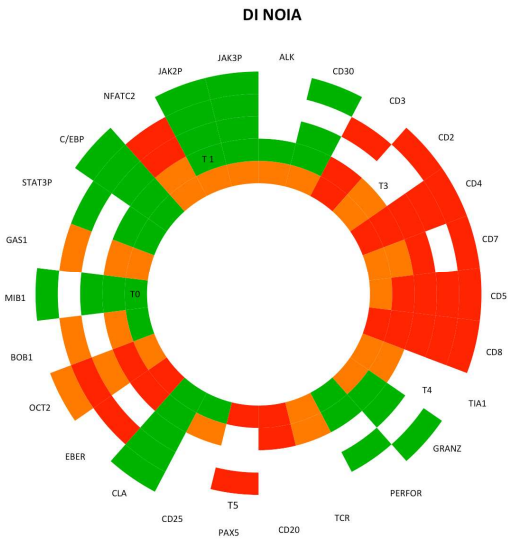
ALCL primary and correspondent ALCL tumorgraft display identical immunoprofiles

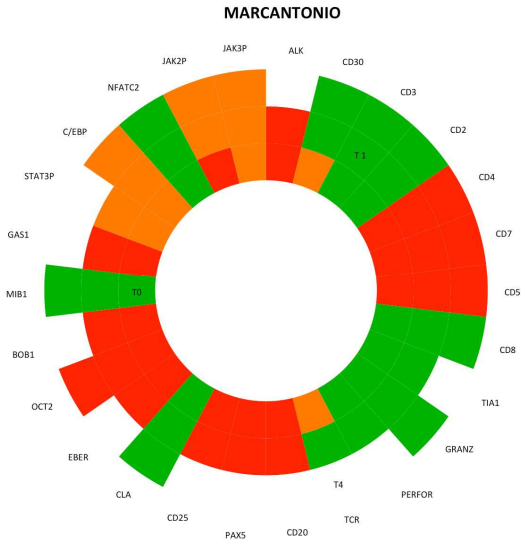
Primary ALCL-1

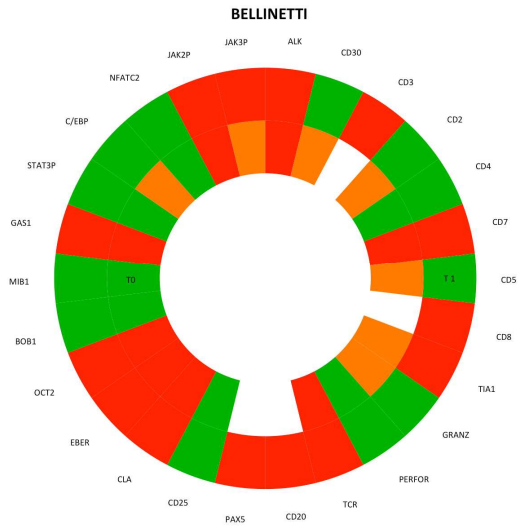


Tumorgraft ALCL-1-T3

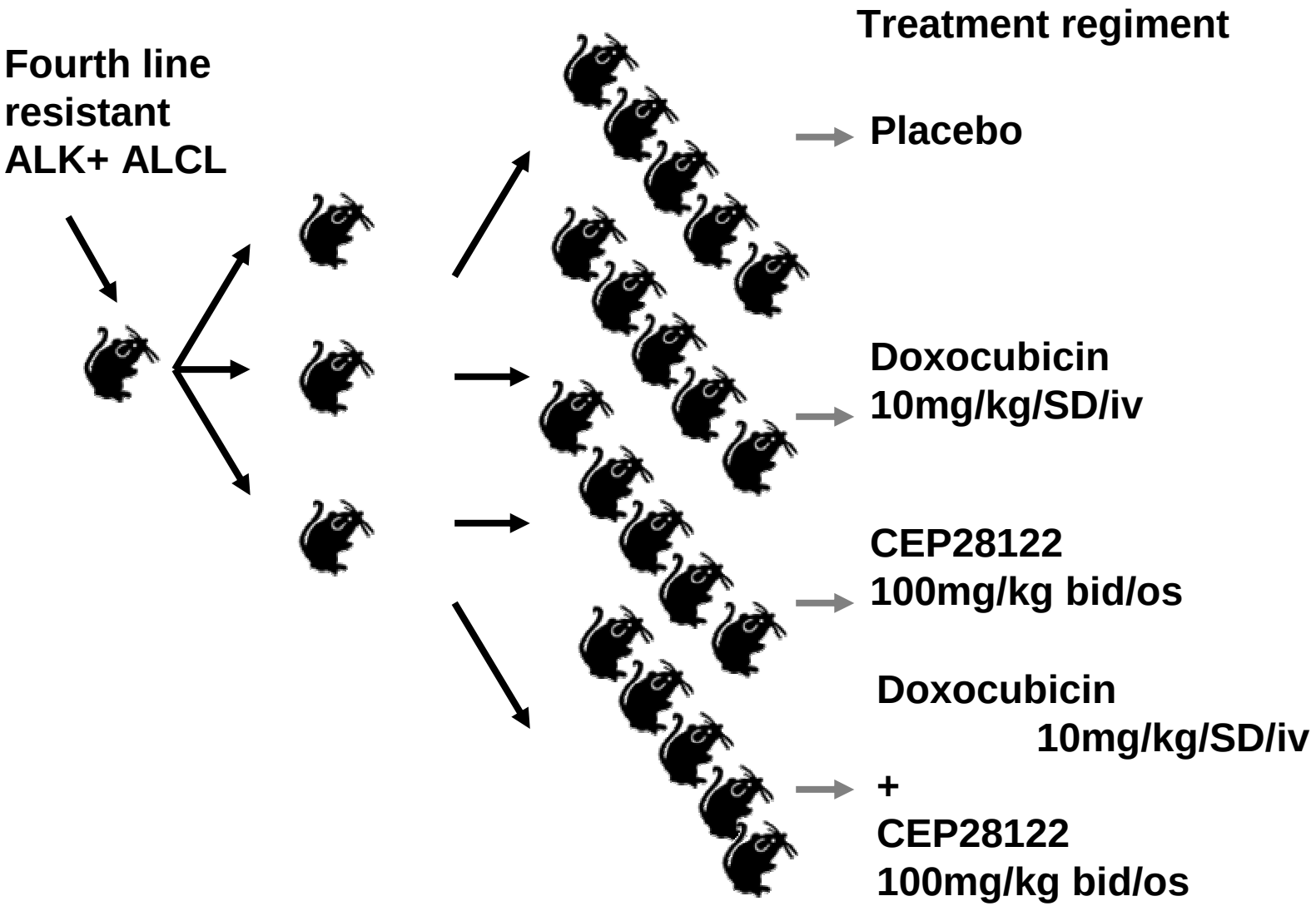




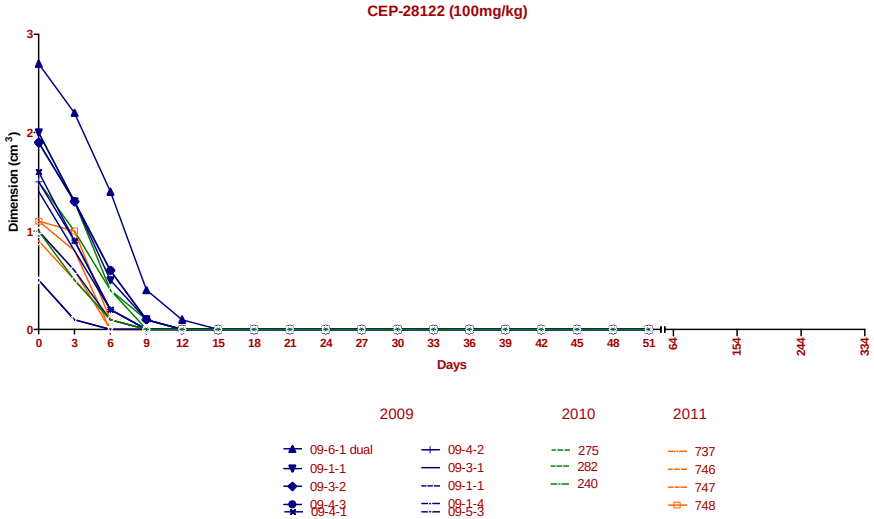


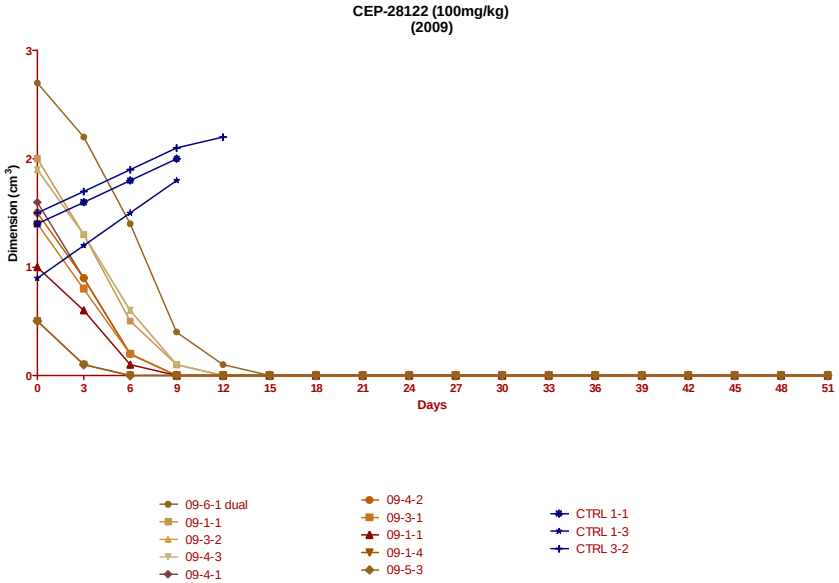


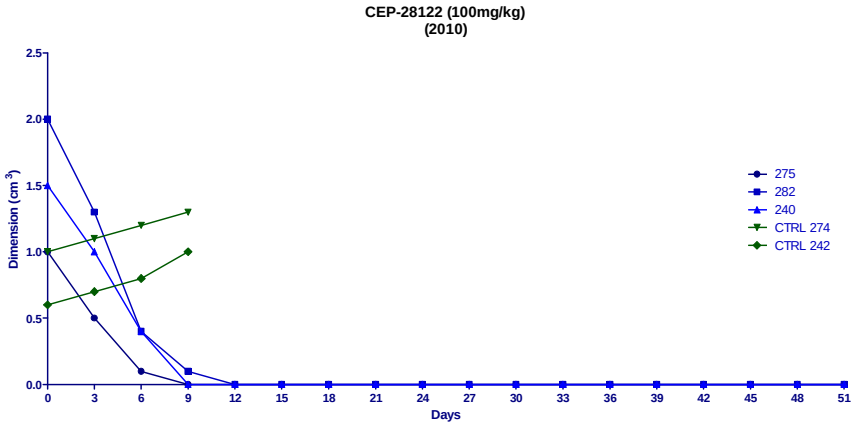
Preclinial therapeutic strategies

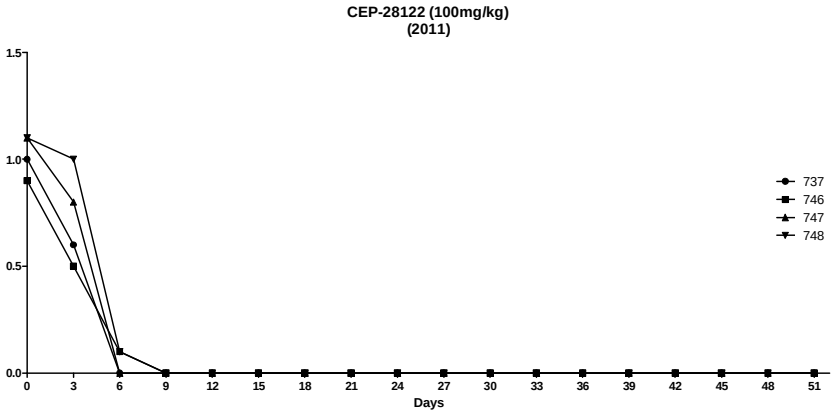


Anti-ALK CEP28122 cures ALK+ ALCL tumorgraft

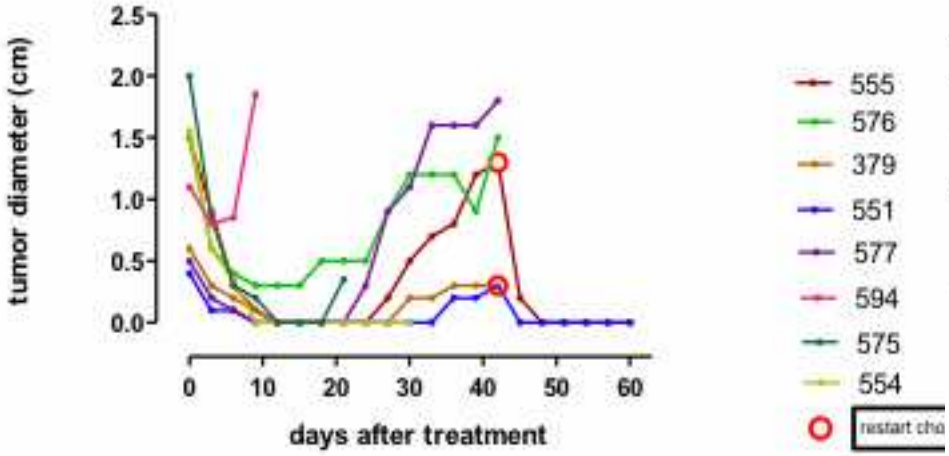




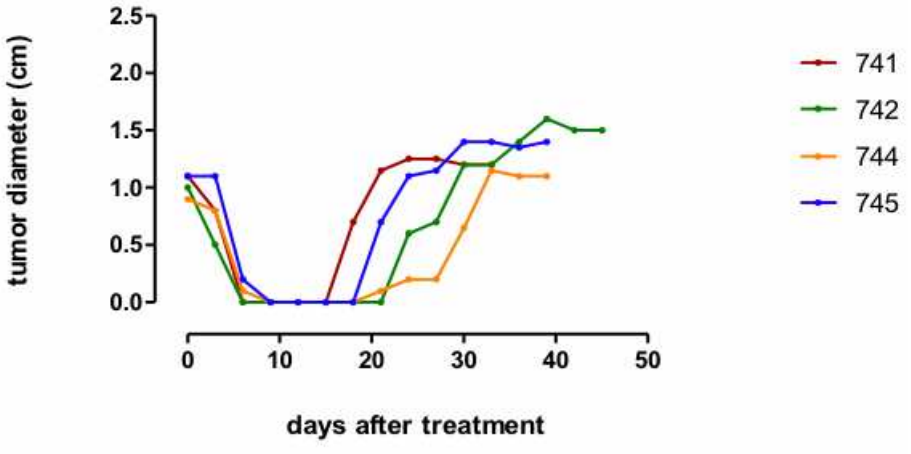




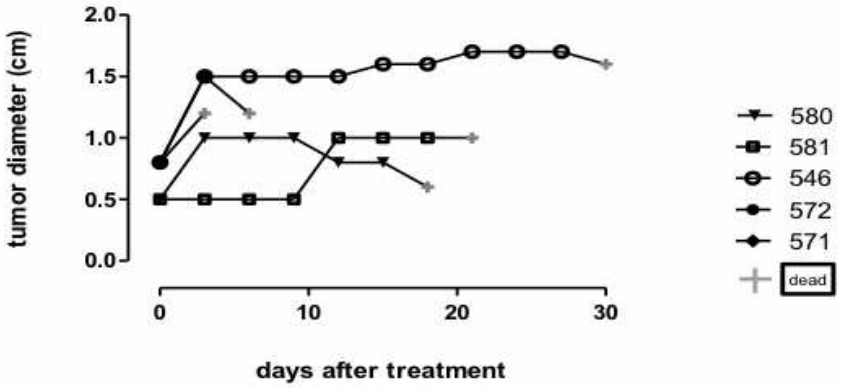
CHOP ALCL-2 1th



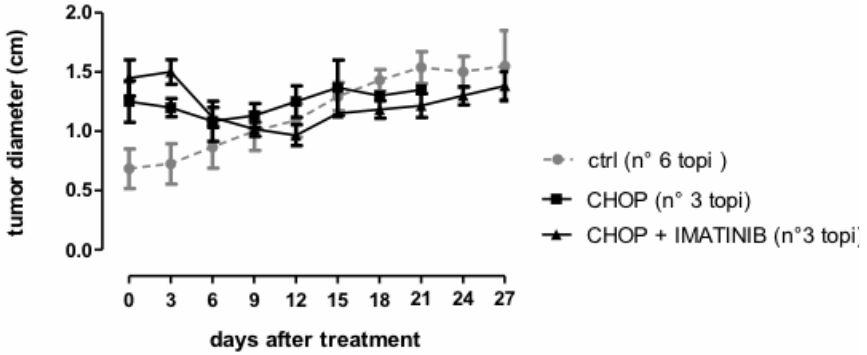
CHOP ALCL-2 2nd



CHOP ALCL-3 1th



CHOP vs Imatinib ALCL-1



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Elisa Pellegrino

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Elisa Ficcara

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Raul Rabadan

Policlinico di Milano

Antonino Neri

Luca Agnelli

MBC

Flavio Cristofani

Guido Forni

Ferdinando di Cunto

Paolo Provero

Silvio Aime

Dario Longo

Fiorella Altruda

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ISIS

James G Karras

Cell Signalig Technology

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